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CONTENTS

NOTE ON THE POSTURE OF THE BIRD'S WING, AND ITS SUPPOSED CONTROL BY SYMPATHETIC NERVES. *O. W. Tiegs* 547

STUDIES IN GASTRIC SECRETION. III. EVIDENCE IN REFUTATION OF THE ROSEMANN THEORY OF HYDROCHLORIC ACID FORMATION. *Franklin Hollander* 551

STUDIES ON THE OVERDISTENDED HEART. I. EFFECTS OF VENESECTION. *W. A. Brams and L. N. Katz* 556

STUDIES ON THE OVERDISTENDED HEART. II. THE RÔLE OF RELAXATION IN FILLING THE DISTENDED AND OVERDISTENDED HEART. *L. N. Katz and W. A. Brams* 569

THE EXCRETION OF URINE IN THE DOG. I. THE UREA AND CREATININE CLEARANCES ON A MIXED DIET. *Norman Jolliffe and Homer W. Smith* 572

STUDIES ON THE URINARY EXCRETION OF OESTRIN, WITH ESPECIAL REFERENCE TO THE EFFECT OF THE LUTEINIZING HORMONE AND PROGESTIN. *George van S. Smith and O. Watkins Smith* 578

THE EFFECT OF THE VAGUS ON THE DIASTOLIC SIZE OF THE HEART AND ITS BEARING ON THE QUESTION OF CARDIAC TONUS. *George Crisler and Edward J. Van Liere* 585

STUDIES IN THE PHYSIOLOGY OF VITAMINS. XVI. THE EFFECT OF EXERCISE ON THE TIME REQUIRED FOR THE DEVELOPMENT OF THE ANOREXIA CHARACTERISTIC OF LACK OF UNDIFFERENTIATED VITAMIN B. *George R. Cowgill, H. A. Rosenberg and J. Rogoff* 589

THE INFLUENCE OF PHYSICAL TRAINING ON THE BASAL METABOLIC RATE OF MAN. *Edward C. Schneider and A. O. Foster* 595

BILE FLOW AND BILE PIGMENT OUTPUT AFTER DENERVATION OF THE LIVER. *Harold Lundberg* 602

STUDIES ON CARBOHYDRATE METABOLISM FOLLOWING DENERVATION OF THE LIVER. *Joseph M. Donald* 605

THE FORMED ELEMENTS OF THE BLOOD, COAGULATION TIME, PEPTONE LEUKOPENIA AFTER SECTION OF THE NERVES TO THE LIVER. *Frederick C. Hill* 610

THE EFFECT OF DENERVATION OF THE LIVER ON SECRETION OF BILE. *Lorin V. Hillyard* 612

HEAT REGULATION AND WATER EXCHANGE. XIII. INSENSIBLE WEIGHT LOSS AND LIVER HYDRATION IN THE ONSET OF COCAINE FEVER. *H. T. Marshall, B. F. Aydelotte and H. G. Barbour* 615

SOME FACTORS REGULATING THE UTILIZATION OF SPLENIC IRON. *S. F. Cook and N. M. Spilles* 626

BLOOD REGENERATION STUDIES. I. CHANGES IN THE VOLUME, NUMBER AND SIZE OF THE ERYTHROCYTES IN HEMORRHAGIC ANEMIA. *Jane M. Leichsenring and Hortense H. Hönig* 636

COMPARATIVE STUDIES ON THE PERIPHERAL AND CENTRAL RETINA. IV. TEMPORAL SUMMATION OF SUBLIMINAL VISUAL STIMULI AND THE TIME COURSE OF THE EXCITATORY AFTER-EFFECT. *Ragnar Granit and William A. Davis* 644

COMPARATIVE STUDIES ON THE PERIPHERAL AND CENTRAL RETINA. V. THE SENSATION-TIME CURVE AND THE TIME COURSE OF THE FUSION FREQUENCY OF INTERMITTENT STIMULATION. *Ragnar Granit and Edward L. Hammond* 654

COMPARATIVE STUDIES ON THE PERIPHERAL AND CENTRAL RETINA. VI. INHIBITION, SUMMATION, AND SYNCHRONIZATION OF IMPULSES IN THE RETINA. *C. H. Graham and Ragnar Granit* 664

THE RELATION OF CORTIN TO THE MAINTENANCE OF BODY TEMPERATURE. *Frank A. Hartman, Katherine A. Brownell and Alford A. Crosby* 674

PUPILLARY DILATATION PRODUCED BY DIRECT STIMULATION OF THE TEGMENTUM OF THE BRAIN STEM. *W. R. Ingram, S. W. Ranson and F. T. Hannett* 687

THE BASAL METABOLISM OF OKLAHOMA WOMEN. *Callie Mae Coons* 692

BASAL METABOLISM IN RELATION TO NUTRITIONAL STATUS. *Callie Mae Coons* 698

CAPILLARY PRESSURE AND HYPEREMIA IN MUSCLE AND SKIN OF THE FROG. *Eugene M. Landis* 704

INDEX 717

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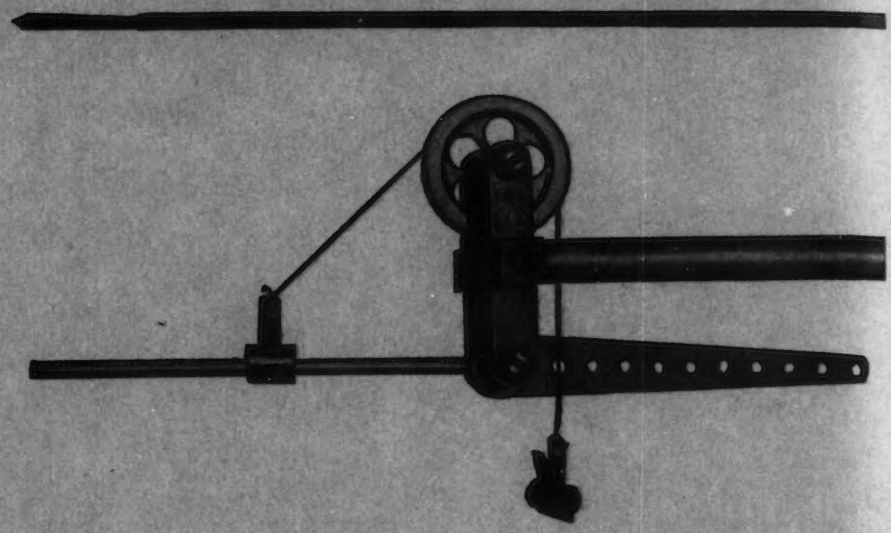

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NOTE ON THE POSTURE OF THE BIRD'S WING, AND ITS SUPPOSED CONTROL BY SYMPATHETIC NERVES

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From the Department of Zoology, University of Melbourne, Australia

Received for publication April 1, 1931

When sympathetic nerves to a limb are severed, due care being taken to avoid injury to the somatic nerves, no perceptible effect is produced upon the posture of the operated limb in the conscious animal (cat, dog, rabbit, goat, bird). This would seem to provide conclusive evidence that the sympathetic nerves play no part whatever in maintaining postural tone.

But it has been urged against this, that any resulting defect in tone is either immediately, or after a longer interval compensated for by the somatic nerves. Such an explanation would account for some of the results that have been reported to follow upon sympathectomy; at the same time there is no very convincing evidence that such compensation actually occurs. The interest of the experiment here described lies in the fact that the possibility of such compensation masking the effects of sympathectomy is excluded.

Hunter (1924) pointed out certain anatomical features of the bird's wing which render it peculiarly suitable for the study of the problem of the relation of sympathetic nerves to muscle tone. The brachial plexus is derived from the lowest cervical nerves (C_{11}–C_{15}); on the other hand, as Langley found, the preganglionic fibres that end in the large sympathetic ganglia associated with the brachial nerves, emerge from the spinal cord through the first two thoracic nerves, i.e., caudal to the brachial plexus. From the first thoracic nerve the sympathetic cord passes forwards as two strands of fibres, a thicker and a thinner, which lie respectively below and above the cervical ribs. Consequently severing the sympathetic cord just below the brachial plexus will eliminate any central control over the postganglionic sympathetic supply to the wing.

Hunter performed this experiment and obtained a marked droop in the wing. Kuntz and Kerper (1926) confirmed this. Coates and Tiegs

(1928) reported that if sufficient precautions were taken to avoid injury to adjacent spinal nerves, the droop did not occur. Subsequently Kuntz (1929) stated that if the operation were done with greater care than in his earlier work, so that injury to the somatic nerves was excluded, no perceptible effect was produced on the posture of the wing. He states, however, "the loss of the influence of the sympathetic innervation in the posture of the bird's wing, as in that of the mammalian limb, is readily compensated in the normal behaviour of the animal, by the cerebrospinal nerves."

In the bird's wing, but not in the mammalian limb, the possibility that such compensation may occur can be excluded. For Trendelenburg (1906) found that the posture of the wing remained unaffected even after afferent impulses arising from the wing itself had been eliminated by dorsal root section; but that the desensitised wing, if drawn down from its normal resting position was not replaced, since the bird did not know that it had been moved. It is therefore possible by desensitising a wing, to prevent the bird from becoming aware of, and therefore compensating for, any droop that may occur when such a wing is also deprived of its sympathetic supply.

The experiment here described consists in cutting the dorsal roots of the nerves to the brachial plexus, severing the sympathetic cord just caudal to the brachial nerves, and observing the effect of such an operation on the posture of the wing. Hunter stated that such an operation left the wing helpless; "the wing was heavy and dependent as in flaccid paralysis." Kuntz and Kerper confirmed this. Coates and Tiegs reported on the contrary that in their experiments the wing thus operated upon showed only a slight droop. The droop was probably due to injury of the cord produced while exposing it; for the cord is very thick in this region and almost completely fills the spinal canal, thus rendering exposure, without injury to it, difficult. But to exclude the possibility that division of the sympathetic cord might be the cause of the droop I have repeated this experiment, taking the greatest possible care not to injure the cord or ventral roots.

The left sympathetic strands, exposed from the dorsal aspect, were cut with fine sharp scissors, without exerting any pull on the adjacent spinal nerves. The spinal canal was gradually opened, and the required length of cord exposed, the utmost care being taken to avoid even touching the underlying nervous tissue; the spinal ganglia of the left brachial plexus were located, and the dorsal roots severed proximal to the ganglia. Fine sharp scissors were used for the purpose, and injury to the cord and ventral roots was scrupulously avoided. Only slight temporary bleeding occurred in cutting through the overlying vascular pia mater.

Half an hour after completing the operation the bird was sufficiently

recovered from the anesthetic to permit examination of the wing. The accompanying photographs were taken a day later. When the bird is standing at rest the wing is held in the normal position, and when compared with the unoperated wing shows no trace of a droop (fig. 1). Moreover the bird can still use the wing in flight. The only perceptible effect of the operation is that the bird ignores any mechanical displacement of the wing (fig. 2). As Trendelenburg showed, such a desensitised wing will remain in any position in which it is put, since the bird has no knowledge that it has been moved; in contrast the normal wing, if drawn down, is smartly flicked into position again.

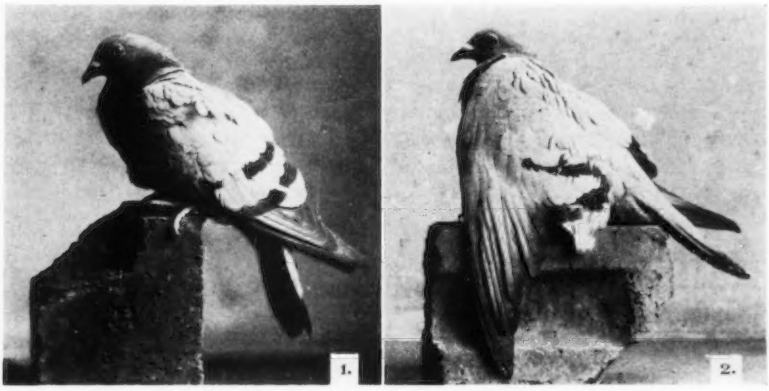

Fig. 1. To show the resting posture of the wing after dorsal root section of the nerves to the left brachial plexus, and division of the preganglionic sympathetic fibres to that wing. The posture of the wing is not perceptibly altered.

Fig. 2. The same bird; the wing has been drawn down to an abnormal position; but the bird makes no effort to restore it.

It may be suspected that a tendency in the wing to droop is being counteracted by compensatory action of spinal nerves in response to afferent impulses produced by the droop, but reaching the cord from some source other than the wing itself, (e.g., eyes or labyrinth). But we have already seen that such impulses, if they occur, are insufficient to restore the desensitised wing to its normal position if it has become displaced.

SUMMARY

1. In the pigeon division of the preganglionic sympathetic fibres to the wing does not produce a droop in the wing even when the possibility of compensation by somatic nerves is eliminated through dorsal root section of the brachial plexus.

2. The existence of a component of postural tone subserved by sympathetic nerves is therefore excluded.

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STUDIES IN GASTRIC SECRETION

III. Evidence in Refutation of the Rosemann Theory of Hydrochloric Acid Formation[1]

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Received for publication April 27, 1931

All of the current opposition to the Heidenhain-Pavlov theory of the constancy of acidity of the parietal secretion has its roots in the opposing hypothesis of Rosemann (1907). This hypothesis, it may be recalled, ascribes the presence of cations in gastric juice to incomplete conversion of neutral chlorides to hydrochloric acid in the parietal cells. The Heidenhain-Pavlov theory, on the other hand, maintains that the source of these cations is not the parietal secretion but other fluids, like the mucous secretion. In paper I of this series (Hollander and Cowgill, 1931), evidence in direct confirmation of the latter theory was adduced. In the present contribution, data will be presented in refutation of Rosemann's major proposition, thus furnishing additional substantiation of the Heidenhain-Pavlov theory.

This proposition (Rosemann, 1907, p. 75) maintains, in direct variance with Pavlov's position, that the acidity of the gastric juice always decreases during the falling off of secretion, even though there is no direct evidence for an increased formation of mucus. The emphasis given this argument by other investigators (Foster and Lambert, 1908; Babkin, 1929) is indicative of the importance which has been attached to it. In the following it will be shown, by reason of an effect overlooked by Rosemann, that observations similar to his are in conformity with the Heidenhain-Pavlov theory.

Method. The present investigation was conducted on stomach pouch dogs of both the Pavlov and the Heidenhain varieties, using the continuous collection procedure in all detail as described in paper I. All of the usual precautions were observed with the utmost care. In particular, all retained juice was drained off previous to stimulation, leaving only a thin coating of mucus on the surface of the mucosa. Secretion was induced

[1] A preliminary report of this work was presented in the Proc. Soc. Exp. Biol. and Med., xxvii, 817 (1930).

by either the subcutaneous injection of histamine or the feeding of any one of a number of food preparations. In all cases, collection of juice was continued until the rate of secretion had again fallen to a value approximately equal to that for the first sample. In plotting the results of experiments such as these, it is the general practice to plot the acidity and rate values against times corresponding to the ends of these collection intervals. However, these figures represent average values throughout the entire period of collection, and therefore they were plotted here at times corresponding to the midpoints of these intervals.

Acidities are all reported in terms of millimoles per liter, though in some of the experiments the values were first determined electrometrically and thereafter converted to millimolar concentration by calculation. This change was made because of the logarithmic nature of pH values and the complication involved in a comparison of such logarithms. In those

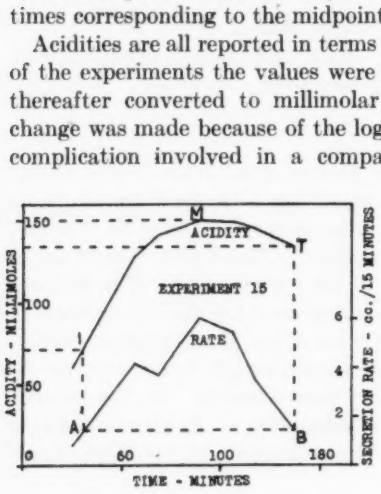

Fig. 1

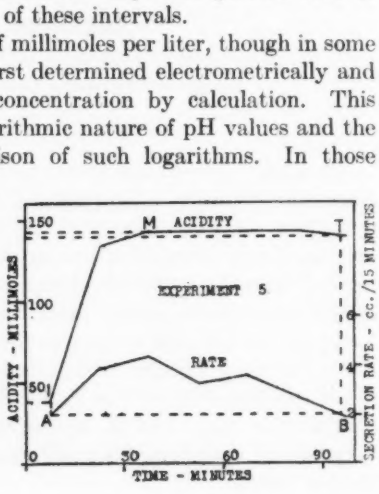

Fig. 2

experiments in which acidity was determined directly by titration, phenolphthalein was used as the indicator. The methods of determinination, both electrometric and titrimetric, and also the formula for the interconversion of pH and molarity values, have been described elsewhere (Hollander, 1931).

Observations. In Rosemann's experiments, the gastric fistula contained a metal cannula which, it has already been pointed out (Hollander and Cowgill, 1931), is conducive to the continuous secretion of mucus. Such a flow of mucus, together with the peptic secretion and other minor factors, will cause a definite though small reduction of acidity throughout the experiment.[2] The initially retained mucus, on the contrary, will

[2] The frequency with which Rosemann reported a red or yellow coloration of the gastric juice in his experiments indicates that blood, and probably an exudate, arising from trauma at the site of insertion of the cannula, also played a part in this action. In our experiments, such effects have been entirely eliminated.

exert its influence only at the beginning. Consequently, during the latter part of the secretory period, we may expect simply a *less marked* depression of acidity than at the beginning, rather than the complete absence of such a reduction. The possibility of such a secondary occurrence of acid-neutralizing material, evidently, was overlooked by Rosemann.

This expectation has been confirmed by some of the present experiments, a typical case of which is illustrated in figure 1. In these experiments, the presence of a fairly large catheter induced an effect similar to the mucus-stimulating action of the metal body in Rosemann's experiments. The occurrence of this irritant action was corroborated by the appearance throughout the experiment of flakes or strings of mucin. In order to compare acidities at the beginning and the end of the secretory period, a horizontal line was drawn from the first point, A, on the rate curve until it met the curve at its descending portion, B.[3] Verticals, drawn through the ends of this horizontal so as to intercept the acidity curve, thus determined the acidities (I and T) at two times during the experiment when the rates of secretion are identical. In the illustration, it can be seen that, although the terminal acidity falls below the highest acidity, M, attained in the experiment, the difference between the two is not large enough to be consequential. On the other hand, the initial acidity, I, is less than half the maximum value.

The data obtained in a series of fifteen experiments with five different dogs have been analyzed in this way. In all cases the results indicate that the acidity at the end of the experiment is not so very much less than the acidity at the height of secretion. The magnitude of this difference, $(M-T)$, was determined in each case; the average value of this difference in the fifteen experiments was 8.5 millimoles (± 5.5), which is less than 5 per cent of the average of all the maximum acidities attained in these experiments. In contrast with this, the average of the numbers representing the difference between maximum and initial acidities, $(M-I)$, equals 67 millimoles or about 47 per cent of the maximum acidity average. These results are in direct agreement with our contention that after the retained mucus has once been washed out, the reduction in acidity, though it may occur, is exceedingly small and may therefore be ascribed to the slight continuous flow of mucus, pepsin secretion, etc.

On the other hand, it was Rosemann's absolute condition for the accept-

[3] In two of the experiments, the volume of juice in the last sample was insufficient for a titrimetric acidity determination. Since the penultimate sample corresponded to a rate somewhat greater than that of the first sample, the method of comparison described above had to be modified. For these two experiments, therefore, the horizontal line AB was drawn from B, the point corresponding to this penultimate sample, to a point A between the first and second samples. The acidity, I, corresponding to this point, determined by a vertical line, AI, was then taken as the "initial acidity."

ance of the Heidenhain-Pavlov theory that only at the beginning of the experiment should the acidity of the gastric juice be lower than at the height of secretion. Now, in some of the present experiments, the conditions were such as to eliminate the continuous mucous flow almost entirely, thus yielding a fluid of the "normal maximum acidity" previously established. In several such cases, the acidity remained constant within the usual limits of error until the very end of the experiment. Such experiments were characterized by the absence of mucin from all samples collected after the first one or two. A typical example of these is given in figure 2, wherein the acidity remains constant for one and one-quarter hours after having attained its maximum value. In four such experiments the values for $M-T$ were 0, 3, 2, and 0, although the average value for $M-I$ was 53 per cent of the maximum acidity.

Therefore, not only are the results in agreement with the demands of the Heidenhain-Pavlov theory with regard to terminal acidities, but the deviations which Rosemann observed have been accounted for. Once the retained mucus has been washed out, the fluid collected is almost pure parietal secretion of unchanging acidity. This constancy of acidity is maintained until the rate of secretion falls to a level below that of the initial rate, i.e., until the flow of mucus and pepsin, in proportion to the flow of hydrochloric acid, is again large enough to become a significant factor. On the other hand, if experimental conditions be such that the flow of mucus, throughout the experiment is fairly large, then a fall in terminal acidity will be observed, but even this drop is negligible when compared with the difference between the initial and maximum acidities.

SUMMARY

According to Rosemann, it is an essential condition for the acceptance of the Heidenhain-Pavlov theory of hydrochloric acid formation, that the terminal acidity in any one experiment be not significantly lower than the highest value attained in that experiment. None of his own observations satisfied this condition. In the present contribution, the terminal acidity is specifically defined for purposes of analysis as the acidity of that sample, the mean secretory rate of which is the same as that of the first sample in the experiment. It is then shown that the terminal drop in acidity is very small, and may be ascribed to a continuous secretion of mucus, pepsin, etc., throughout the experiment. Further, when the experimental conditions are such as to reduce this continuous secretion to a minimum, the terminal drop disappears; i.e., the constancy of acidity which Rosemann demanded as an essential consequence of the older theory can be demonstrated. Thus, the major argument on which Rosemann based his opposition to the Heidenhain-Pavlov theory is refuted.

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STUDIES ON THE OVERDISTENDED HEART

I. Effects of Venesection[1]

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Received for publication June 8, 1931

Venesection, for centuries considered a panacea for nearly all human ailments, has lost its prestige during the last fifty years in spite of occasional attempts to revive its usage. This is due in great part to a lack of adequate studies on the dynamic effects of blood letting and the various compensatory mechanisms which act shortly after venesection and tend to modify the effect of the reduction in return flow. Such complications as the anoxemia resulting from the bleeding, on the one hand, and the rapid restoration of the blood volume by migration of fluid from the tissues to the vascular bed, on the other—which is well established—are perhaps the most important ones. These and other mechanisms are further modified by the condition of the heart and circulation and the results may very well be different in the normal and overdistended heart. Recently studies by Middleton (1929) have led him to recommend the procedure in congestive heart failure with excessive venous pressure. Similar favorable impressions are also reported by Eyster and Middleton (1927), Cumston (1925), Clark (1925), Kolbert (1927), Rosenow (1926), and Bürger (1925). The chief objection to most of this work is that other measures were being used at the same time so that the actual usefulness of venesection alone was not evaluated sufficiently accurately. While improvement was reported, this was frequently transient and the ultimate course of the condition was but little affected. Thus, for example, Middleton reported that about half of his cases died in the hospital.

While considerable experimental work has been done on the effects of venesection or arterial bleeding in animals practically none has been done on the effect of venesection on the overdistended, non-diseased heart of an experimental animal. In the present research an attempt was made to compare the effects of venesection on the non-distended and ovendistended

[1] Aided by the Emil and Fanny Wedeles Fund of the Michael Reese Hospital for the Study of Diseases of the Heart and Circulation.

non-diseased hearts with the idea that such an analysis was a necessary step in the evaluation of the possible clinical benefits of venesection. At the same time it is realized that the overdistention was produced acutely and that the experimental conditions differed in this, as well as in many other respects, from clinical congestive heart failure with excessive venous pressure and a dilated heart.

In these experiments, the heart was considered overdistended when it showed one of the following changes: viz., a decrease in volume amplitude with an increased diastolic size, a decreased pressure amplitude with the diastolic initial pressure elevated, a decrease in the blood pressure level and pulse pressure as the heart was distended. It is well known, following the work of Starling (1915), that increasing the size of the heart (its diastolic volume and pressure) up to a certain point improves its contraction, but an optimum is reached beyond which increasing the size depresses contraction. This is the overdistended heart. Clinically a similar distinction may be made between the physiologically and pathologically dilated heart.

METHOD OF STUDY. Dogs anesthetized with morphine and sodium barbital were used. The thorax was opened and respiration was maintained artificially. Simultaneous records were obtained on bromide paper of the right and left intraventricular pressure pulses, and of the aortic and pulmonary arterial pressure pulses. For this purpose Wiggers' manometers (1928) were inserted into each ventricular cavity or through the arterial walls with the cannulae placed within 1 cm. of the semilunar valves. Simultaneous curves were recorded without parallax by projecting the beams of light from a duo-slit lamp (Katz and Baker, 1924) onto the mirrors of the manometer which reflected them into the photokymograph.

Records were obtained also of the volume changes on a smoked drum using the arrangement described by Wiggers and Katz, 1922. Mean arterial blood pressure was followed in some experiments as well as the mean pressure in the superior vena cava and pulmonary veins.

The venesection was made on the cephalad end of the external jugular vein. The quantity of blood withdrawn, which averaged 100 cc. in the non-distended and 150 to 200 cc. in the overdistended heart, was computed to be roughly equivalent to the amount removed in clinical venesection. The heart was distended and overdistended either with defibrinated blood, physiological saline or a mixture of both. In a few experiments a 6 per cent gum arabic solution in 0.9 per cent sodium chloride was used for infusion.

RESULTS. *1. Volume changes.* Without exception and in accord with previous workers we found a progressive decrease in the diastolic, systolic, and stroke volumes following venesection in the non-distended heart; after bleeding was stopped there was a variable tendency for the output and diastolic size of the heart to increase again.

While it was easy to distend the heart by transfusion it was not so easy in many experiments to overdistend it.[2] A "steady state" was reached in some animals where the size and output of the heart did not change for a long time. This "steady state" can be accounted for by the presence of a more or less perfect balance between the escape of fluid from the blood stream and the increase of the capillary bed (Meek and Eyster, 1922), on the one hand, and the inflow of fluid on the other.

The effect of venesection on the overdistended heart was not constant. Occasionally, as in figure 1, venesection, which was begun just before *12*, improved the heart previously overdistended, as evidenced by an increase in

Fig. 1. Ventricular volume curve showing beneficial effect of venesection on overdistended heart. Overdistention starts at *11* and venesection starts just before *12*. Diastolic volume is top of curve and systolic volume, bottom. An increase in volume is shown by a rise of curve. Time at top is in 5 second intervals.

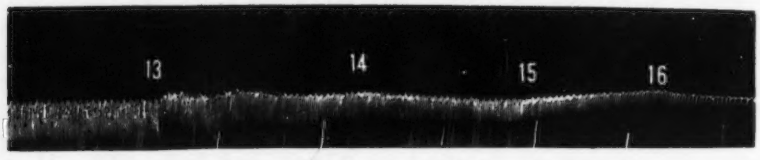

Fig. 2. Ventricular volume curve showing lack of benefit of venesection on the overdistended heart. Curve recorded as in figure 1. At *13* overdistention starts: *14* is venesection; *15*, further transfusion; and *16*, another venesection.

the stroke volume of the ventricle as the diastolic size of the heart decreased. More often venesection had no beneficial effect on the heart. For example, in figure 2, taken later in the same animal from which figure 1 was obtained, the progressive decrease in the systolic output of the heart, initiated by overdistention (indicated by *13*) continued during the venesection (begun at *14*) and during a subsequent transfusion (started at *15*) and later venesection (begun at *16*) until the animal's death. It would seem, therefore, that while venesection may sometimes be beneficial to an

[2] The criterion used to determine when the heart was overdistended was a decreased stroke volume accompanying an increase in diastolic volume.

overdistended heart, there is also danger that progressive failure of the heart may be unchecked and actually accelerated by the procedure.

2. *Changes in the simultaneous pressure levels within the right and left ventricle.* The criterion used to determine when the heart was overdistended was a decrease in the pressure maximum and pressure amplitude accompanying a rise in initial pressure, e.g., compare segment *A* (distended) with segment *B* (overdistended heart) in figures 3 and 4. In about half the experiments venesection of such overdistended hearts led to a further

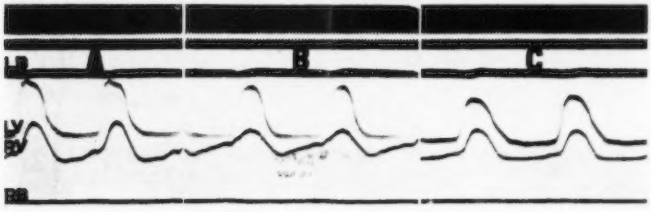

Fig. 3. Simultaneous right (*R V*) and left (*L V*) intraventricular presure curves showing effect of venesection on overdistended heart. *R.B.* and *L.B.* base lines for right and left ventricular curves, respectively. Time at top, each double vibration equals $\frac{1}{30}$ of a second. Segment *A* is distended heart; segment *B*, overdistended heart; segment *C*, immediately after venesection.

Fig. 4. Simultaneous right and left intraventricular pressure curves showing effect of venesection on overdistended heart. Time and curves as in figure 3. Segment *A* is distended heart; segment *B*, overdistended; segment *C*, immediately and segment *D*, some time after venesection.

immediate drop in the pressure amplitude, a maximum of 29 mm. Hg in the left and 17 mm. Hg in the right ventricle. In the remainder of the experiments the pressure amplitude rose immediately following venesection, viz., figures 4 and 5; the maximum rise being 17 mm. Hg in the left and 13 mm. Hg in the right ventricle. As a rule, the changes in pressure were more marked in the left ventricle than the right, viz., figure 5, regardless of the direction in which they changed. Occasionally, as in figure 4, the reverse was true. In only one experiment out of thirteen, viz., figure 3, were the changes immediately following venesection in opposite directions.

Venesection in the overdistended heart did not always lead to a rise in maximum pressure when the pulse amplitude rose. Sometimes the maximum pressure remained unchanged (viz., right ventricle, fig. 3, segments *B* and *C*, left ventricle, fig. 5, segments *A* and *B*), or actually fell slightly (viz., right ventricle, fig. 5, segments *A* and *B*). In several of the experiments, however, the maximum pressure rose following venesection (viz., both right and left ventricle, fig. 4, segments *B* and *C*), indicating that the force of the heart was actually sufficiently improved to overcome the effect of the drop in both systemic and pulmonary arterial blood pressures.

In those experiments where the pressure amplitude fell immediately following venesection, the drop continued. In most of the experiments in which the pressure amplitude increased immediately after venesection, the improvement persisted or increased in the next twenty minutes, cf. figure

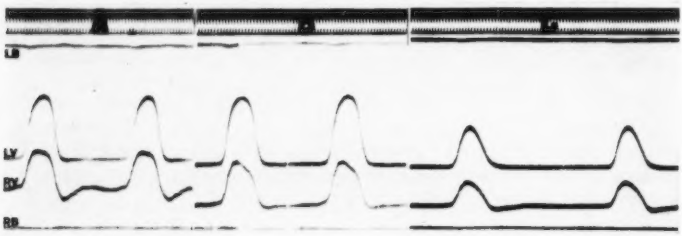

Fig. 5. Simultaneous right and left intraventricular pressure curves showing effect of venesection on overdistended heart. Time and curves as in figure 3. Segment *A*, overdistended heart; segment *B*, immediately and segment *C*, later stage after venesection.

4 (segments *C* and *D*). In one or two of the latter the improvement was only temporary, and was succeeded by a depression which was progressive, viz., figure 5 (segments *B* and *C*).

The improvement in the force of the previously overdistended heart following venesection, as evidenced by the increase in pressure excursion and maximum pressure, is in striking contrast to the depressant action of venesection observed in the non-distended heart, where the pressure excursion and maximum pressure almost invariably dropped, usually with some later recovery. However, it must be borne in mind that both the volume and pressure curve studies show that the beneficial effect of venesection is not a consistent result but is often actually replaced by a detrimental effect as regards the output and force of the heart.

Accompanying the immediate decrease in amplitude and maximum pressure in the two ventricles, we expected *a priori* a drop in their initial pressure (the pressure existing in the ventricles just before the onset of systole)

after venesection in the non-distended heart. While such a change occurred in most of the experiments, at least in one chamber, in several experiments the initial pressure remained unchanged or rose in one or the other chamber.[3]

Contrary to the variable changes found after venesection in the non-distended the immediate effect of venesection in the overdistended heart was to reduce the initial pressure consistently, viz., figures 3, 4 and 5, a decrease which was usually greater in the left ventricle than in the right, although occasionally the reverse was found. In the few experiments where venous pressure readings were made, a similar drop in pressure was noted in both the superior vena cava and pulmonary veins following venesection in the overdistended heart. In most of the experiments the pressure drop was greatest in the pulmonary veins.[4] The explanation for the greater drop in pressure on the left side may be due to the higher elasticity coefficient of the thicker left ventricle. It may, however, express the greater dependence of the filling pressure in the left side on the activity of the right and left ventricles.

In those experiments on the overdistended heart in which the changes were followed for some time, the fall in initial pressure continued, probably because of oozing of blood from the smaller cut vessels, or of osmosis since a mixture of low osmotic pressure was used in the infusion.

The improvement of the heart's condition as the initial pressure (and diastolic volume) dropped suggests that the depression in these overdistended hearts is, in part at least, due to the mere mechanical overdistention. It is on this basis only that the relief of overdistention clinically is worth attempting. Our results further suggest that if venesection is unsuccessful in improving the heart in about half of these acute experiments it may be equally unsuccessful in clinical conditions with cardiac overdistention in which other factors probably play a part. The results raise the question whether, from the clinical standpoint, venesection should be repeated if it is not followed by definite improvement the first time it is performed.

3. *Changes in the aortic and pulmonary arterial pressures.* The changes

[3] Some of this discrepancy may be due to the difficulty of establishing the zero level of the manometers with accuracy. Tests showed that variations of 1 mm. occur by realignment of the carbons in the projecting lamp, and will amount to as much as 2.5 mm. Hg in the left ventricle and 1.0 mm. Hg in the right with the sensitivity employed. Changes within this range were considered within the experimental error and were labelled "no change." A much more serious error, it seems to us, is the shift of the mouth of the manometer cannula as the size of the heart is changed. Errors such as these may explain the bizarre changes in initial pressure which we obtained in some experiments.

[4] It is interesting to observe that we obtained venous pressures during overdistention as high as 445 mm. of saline which agree with the initial pressures also observed of up to 58 mm. Hg.

in the pressure pulses were followed only four times after venesection in the non-distended heart. A drop in systolic, diastolic and pulse pressure was always found following venesection. This was many times greater on the left side than on the right, cf. experiment 18 in table 1. In one experiment where the later effects were followed, a tendency for the systolic and pulse pressures to rise was observed (cf. periods 3 and 4 of expt. 18, table 1). It is interesting that the pulmonary pressure is more stable than the aortic under these conditions of venesection as was observed in other conditions (cf. Katz and Wiggers, 1927; Katz and Siegel, 1929).

TABLE 1

Effect of venesection on the aortic and pulmonary arterial pressures in the non-distended and overdistended heart

	RATE PER MINUTE	AORTIC PRESSURE			PULMONARY ARTERIAL PRESSURE		
		Diastolic	Systolic	Pulse	Diastolic	Systolic	Pulse
		Expt. 18					
	mm. Hg	*mm.* Hg	*mm.* Hg	*mm.* Hg	*mm.* Hg	*mm.* Hg	*mm.* Hg
2. Control	150	106	169	63	16	70	54
3. Venesection	158	89	133	44	14	30	16
4. 5 min. later	146	89	141	52	14	34	20
6. Distention	103	89	193	104	32	83	51
7. Overdistention	111	83	169	86	26	69	43
8. Venesection	125	85	148	63	17	34	17
9. 10 min. later	127	78	141	63	14	30	16
		Expt. 23					
2. Control	176	81	102	21	27	45	18
5. Distention	125	105	181	76	57	114	57
6. Overdistention	102	92	160	68	50	93	43
7. Venesection	120	81	158	77	55	114	59
8. Further venesection	115	83	160	77	50	114	64
9. 2 min. later	115	119	205	86	50	102	52
10. 10 min. later	136	122	194	72	45	79	34

Venesection in the overdistended heart[5] caused little or no change in the diastolic pressure of the aorta. In one case, however, shown in figure 6, the diastolic pressure fell. The pulmonary diastolic arterial pressure dropped immediately in most of the cases, cf. periods 7 and 8 of experiment 18 in table 1. Occasionally, as in figure 6 (cf. segments *C* and *D*), a rise occurred, or the pressure remained unchanged (cf. periods 6, 7 and 9, expt. 23, table 1).

[5] Overdistention was determined by a persistent drop in systolic and pulse pressure as the heart was distended.

The changes in systolic pressure were also quite variable. Thus in experiment 18, table 1 (cf. periods 7 and 8) the systolic pressure fell on both sides. A change in one side was in several instances associated with no change in the other or with a slight drop. Thus in experiment 23 (cf. periods 6, 7, and 8) the systolic pressure rose on the right side and remained unchanged in the left. In figure 6 (compare segments *C* and *D*) it fell on the left and rose on the right. Similar variable changes in the pulse pressures were also observed.

The variability of these results is understandable since, as we pointed out above, the effects of venesection are diametrically opposite in the overdistended heart in different experiments. In some it has a detrimental effect; in others it has a beneficial effect. The fact that the action is not

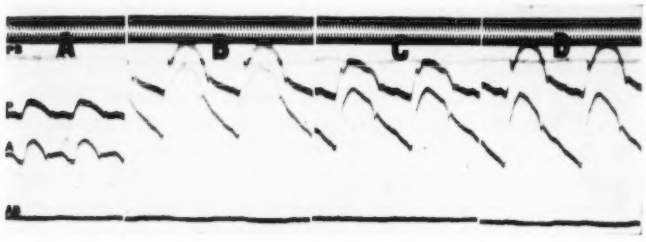

Fig. 6. Simultaneous aortic, *A* and pulmonary, *P*, arterial pressure curves showing effect of venesection on overdistended heart. *PB* and *AB* are base lines of the pulmonary arterial and aortic curve, respectively. Time as in figure 3. Segment *A* is non-distended heart; segment *B*, distended; segment *C*, overdistended; and segment *D*, immediately after venesection.

quantitatively parallel in the two sides indicates that the relief of overdistention by venesection is not parallel in them. We have had several experiments, such as the one shown in figure 3, where the overdistention was more marked in one side than the other. In such experiments venesection might be expected to have unequal and at times even opposite effects on the arterial pressures of the two sides.

As a rule in these particular experiments the diastolic, systolic, and pulse pressures fell in the next twenty minutes (cf. periods 8 and 9, expt. 18, table 1). This is probably to be attributed to the continued oozing out of blood from the circulation. It may well be that more permanent improvement might have been found had we lessened the escape of fluid from the blood stream by using a perfusate of higher osmotic pressure.

Contrary to the effects of venesection in the non-distended heart, transfusion and venesection of the overdistended heart produced noticeable changes in pulmonary arterial diastolic pressure. Apparently the mecha-

nisms keeping pulmonary arterial diastolic pressure constant do not operate so well when the quantity of circulatory fluid is large.

4. *Changes in heart rate.* The changes in heart rate noted following venesection in the non-distended heart were variable. A fall in rate, sometimes marked, occurred eight times; a speeding up in rate, seven times; and no change, six times immediately after venesection. In eleven out of fourteen experiments the rate fell later; in all but two of these, to a level below the resting rate. In the other three experiments no change in rate occurred. The slowing noted is probably a direct effect of the ischemia accompanying the drop in blood pressure acting either on the vagus center or directly on the sinus node. It is interesting to observe that the greatest slowing occurred in those hearts which became poor in condition. The increase in rate observed in some experiments may be due to a depressor reflex.

Slowing was approximately as frequent an immediate result of venesection in the overdistended heart (11 out of 24 experiments), as in the non-distended one. However, slowing was a less frequent finding later after venesection in the overdistended heart, viz., only twice out of 10 experiments in which the changes were followed for 20 minutes. In the majority, the rate speeded up so that it became faster than normal. This striking difference between the later effect of venesection in the non-distended and overdistended heart is probably related to the improvement in the heart's condition found in the latter following venesection. The greatest slowing following venesection in the overdistended, as in the non-distended heart, accompanied obviously dying hearts.

These results suggest that a persistent fall in heart rate after venesection may be a sign of danger while a sustained or moderately increased heart rate may signify that venesection is beneficial. The applicability of these interpretations should be checked clinically.

5. *The asynchronism of the onset of contraction in the two ventricles and the duration of their ejection phases.* There was an asynchrony of the onset of the pressure rise in the two ventricles in the majority of the eight animals where this was studied. Bleeding usually altered the relations of the rise of pressure in the two ventricles in the majority of cases augmenting the asynchrony. Distention of the heart lessened the asynchrony in the majority of cases, while bleeding after overdistention increased it again. These results are in line with those reported by Katz (1925).

The duration of the elevation of the pressure curve was longer without exception in the left ventricle regardless of the distention of the heart, and this difference in straddle persisted after venesection.

Similar measurements of the asynchrony of the onset and end of ejection phase showed that, in all but one experiment out of seven, ejection started first in the right heart. The asynchrony at the end of ejection was more

variable. In the majority of cases the duration of ejection was longer in the right heart. Distention decreased the asynchrony of the ejection periods, as a rule, the duration of the ejection period being consistently prolonged. Venesection of the overdistended heart, on the other hand, abbreviated the ejection period of both ventricles and tended to aggravate the asynchronism of the onset and offset of ejection. The greatest abbreviation occurred when the heart was moribund.

It is noteworthy that while the period of elevated pressure is longer in the left ventricle the duration of the ejection period is shorter in this chamber. In other words, the periods of isometric contraction and relaxation are shorter in the right than in the left ventricle. This is easily explanable on the smaller difference of pressure existing between the right auricle and pulmonary artery as compared with the left auricle and aorta.

6. *Changes in the electrocardiograms.* Electrocardiograms were recorded in 11 animals using the three standard indirect leads, the electrodes being sewed in place subcutaneously. Some of the animals were breathing naturally with chest unopened, others had chest open and artificial respiration, with or without optical manometers in place. Control records were taken before venesection, from 2 to 10 minutes after venesection in the non-distended heart, during overdistention, and shortly after venesection in the overdistended heart.

The most significant changes were noted following venesection when the heart was depressed. Of the ten dogs in whom the electrocardiogram was studied in the non-distended heart, five showed very little change in the electrocardiogram, and in these five the heart rate increased after venesection. In the remaining five a negative S-T, inversion of T, or both were observed. In all but one of these latter five animals a slowing in heart rate was also immediately seen. In two animals there was a typical cove-shaped T wave, in one of these associated with a high take-off of the S-T. In one, the QRS became prolonged and the record resembled a curve of complete bundle branch block. In another, the whole R-T segment was inverted in leads 2 and 3 and became practically monophasic. Extrasystoles were found in one instance. These observations fall into line with previously reported experiments on asphyxia, bleeding and venesection (Gruber, 1930; Feil, Katz, Moore, and Scott, 1931). There can be no doubt that in our experiments the changes were a direct expression of a depressed heart. The resemblance of some of these curves to coronary artery occlusion confirms the impression that acute myocardial ischemia, regardless of etiology, is responsible for these changes in the electrocardiograms.

Transfusion, up to and beyond the original blood volume, produced, in all but one of seven animals studied, a decrease of the inversion of the T wave or changed an inverted T to an upright T. In one experiment a nega-

tive S-T, which appeared during venesection, disappeared after transfusion. These changes are to be anticipated since transfusion improves the heart's activity and increases the circulation of the coronaries.

Venesection of the overdistended heart caused similar though, on the whole, less marked changes in the R-T segment than venesection of the non-distended heart. In only one out of six animals did a negative S-T develop. However in five out of six animals the T wave became inverted. These milder changes are to be anticipated since venesection of the overdistended heart leads to improvement on occasion and only at times has it as deleterious an action as bleeding in the nondistended heart.

SUMMARY

1. The effect of venesection on the non-distended and on the previously, acutely, overdistended heart of the anesthetized dog were compared. The overdistention was brought about by transfusion of defibrinated blood, physiological saline, gum acacia solution, or by a mixture of the former two. The analysis was based on a study of graphically recorded volume curves, on optically recorded synchronous pressure pulses from the two ventricles, and from the aorta and pulmonary artery, on the pressure levels in the systematic and pulmonary veins, and on the electrocardiogram recorded by the three standard indirect leads.

2. Venesection in the non-distended heart caused a reduction in stroke volume of the ventricles, a decrease in the maximum pressure and pressure excursion of the ventricles during systole and a fall in the pressure level in the aorta and pulmonary artery. These changes are in accord with previous work on the effect of hemorrhage. The changes were more marked in the left ventricle and aorta. The stability of the diastolic pressure in the pulmonary artery was striking.

The subsequent course of events, which was followed for twenty minutes, was variable. In some experiments there was a progressive decrease in the pressures and stroke volume; in others there was a tendency for the pressures and stroke volume to increase again; in a few there seemed to be a stabilization of the heart's activity at the new level.

3. Venesection produced a different set of events in the acutely overdistended heart. In many instances venesection led to a further drop in stroke volume of the ventricles, a further decline in the maximum pressure and the pressure excursion of the ventricles during systole, and a further fall in the pressure level and pulse pressure in the aorta and pulmonary artery. In every one of the experiments in which this initial depression of the heart's action was noted, a further progressive depression was observed in the following periods up to twenty minutes. No instance of a secondary augmentation of the pressure levels or excursions was noted, probably because of the escape of the fluid (whose osmotic pressure was

low) from the blood stream. The changes were more marked in the left ventricle but, unlike the non-distended heart, venesection produced, as a rule, a greater drop in the diastolic pressure of the pulmonary artery than in the aorta.

In some experiments venesection overcame the depressing action of overdistention, leading to a temporary or lasting improvement, at least up to twenty minutes. This improvement was evidenced by an augmentation of the stroke volume, by an elevation of the maximum pressure, and pressure excursion of the ventricles during systole, or both, and by a rise in the systolic and pulse pressures in the aorta and pulmonary artery, occasionally accompanied by a rise in the diastolic pressure level and by a fall in the pressures in the systemic and pulmonary veins.

4. Venesection in the previously overdistended heart was always associated with a decrease in diastolic volume and in initial pressure, the drop in the latter being, as a rule, greater in the left ventricle. In the non-distended heart, bizarre variable changes were noted in the initial pressure although the diastolic volume consistently dropped. These bizarre changes in initial pressure are ascribed to artefacts inherent in the experimental method which are sufficiently large to mask occasionally the true changes in the non-distended heart but not the larger changes in initial pressure following venesection in the overdistended heart.

5. The results reported indicate that venesection has a different dynamic effect on the acutely overdistended heart than on the non-distended one. In many instances an improvement in the dynamic action of the overdistended heart can be demonstrated, in others, however, the depression of the overdistended heart's pumping ability is apparently made progressively worse by venesection.

It is realized that the acutely overdistended heart in these experiments is not strictly comparable with the overdistended heart encountered clinically, and that such factors as the presence of myocardial pathology and chronicity of the stasis and its effects, may alter the response to venesection. With these reservations, it is believed that considerable information has been gained in these experiments which may lead to a better evaluation of the clinical possibilities of venesection.

6. The changes in heart rate following venesection were variable both in the non-distended and overdistended heart. The effect of these rate changes on the dynamics of the heart were evaluated in making the analysis of the results of venesection. Temporary or permanent slowing was as frequent a finding as acceleration. Slowing was usually but not always associated with a depression of the heart's dynamic action and with electrocardiographic evidence of intraventricular block or damage. The slowing in these cases was a sinus bradycardia. In extreme form it preceded the death of the animal. These observations suggest that a slowing

up of the heart rate following venesection should be considered a sign of danger. The applicability of this test should be tried clinically.

7. An asynchrony in the pressure rise of the curves obtained from the two ventricles was observed, as well as in the onset and end of the ejection period. Some changes in the asynchrony were noted as the condition of the heart altered. The straddle of the left ventricular pressure curve was found to be consistently longer than the right, although the difference in the straddle of the two curves varied. On the other hand, the duration of ejection was longer, as a rule, on the right side, indicating that the periods of isometric contraction and relaxation were shorter in the right ventricle. The duration of ejection of the two ventricles decreased after venesection and increased on distention of the heart. No consistent changes in the duration of ejection were found when the heart was overdistended. The greatest abbreviation of ejection occurred in the moribund heart.

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STUDIES ON THE OVERDISTENDED HEART

II. The Rôle of Relaxation in Filling the Distended and Overdistended Heart[1]

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Recently experiments on the isolated turtle heart showed that the ventricle during relaxation exerted an aspirating action in the sense that it lowered the pressure in its chamber below that in the filling system (Katz, 1930). In the course of the research reported in part I, it was observed that when the heart was distended or overdistended a diastolic dip, similar to the one seen in the turtle experiments, became apparent in every record of the right intraventricular pressure pulse and on one or two occasions in the left ventricular curve also. Thus in segment *A* of figures 4 and 5 it will be noticed that the foot of the pressure decline in the left ventricular curve is followed by a shallow diastolic dip. In figure 4 this is in turn followed by a gradual rise in the curve during the rest of diastole. A much more noticeable diastolic dip is present in the right ventricular curve in segments *A* and *B* of figures 3 and 4 and in all the segment of figure 5.

Analysis showed that this diastolic dip was greatest in the left side, when it was observed, at the time when the vigor of this ventricle was at a maximum, viz., when it was distended and before it was overdistended. In the right ventricle the maximum diastolic dip occurred while the heart was overdistended and its systolic pressure development decidedly decreased. Venesection decreased this diastolic dip. The presence of the diastolic dip led on occasion to a diphasic appearance of the pressure curve, viz., in segment *B* of figure 3. Our results show that the diastolic pressure dip is more variable than the pressure excursion during systole. As in the turtle heart the minimum pressure in the ventricle was never found to go below atmospheric pressure. When the heart rate was rapid it was not easy, at times impossible, to determine whether the diastolic dip was real or apparent because of the subsequent rise of pressure caused by the

[1] Aided by the Emil and Fanny Wedeles Fund of the Michael Reese Hospital for the Study of Diseases of the Heart and Circulation.

auricle. In the figures used to illustrate this report (viz., figs. 3, 4, and 5) it is not difficult to differentiate the diastolic dip from the subsequent auricular rise as they are separated by a more or less horizontal stretch of the curve.

To rule out the possibility that this dip is an artefact resulting from the presence of two cannulae in the heart chambers the experiments were repeated using only the right ventricular manometer. The heart was slowed by stimulating the vagus in order to separate the auricular effect from the diastolic dip. Figure 7 shows the results in one such experiment. Segment *A*, the control, shows no diastolic dip and only a barely discernible auricular wave. Segment *B* shows the distended heart with a clear-cut diastolic dip and auricular wave distinct from each other. Segments *C* and *D* show the persistence of the diastolic dip while the pressure development is lessened as the heart was overdistended. Segment *E* shows the disappearance of the diastolic dip after venesection. The diastolic dip is

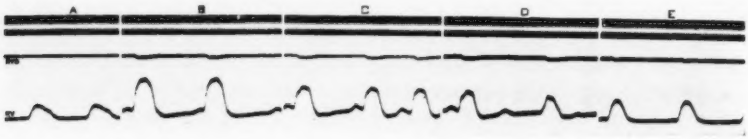

Fig. 7. Right ventricular pressure curve, *RV*, and base line, *RVB*, to show the appearance of a diastolic dip as the heart is distended; time as in figure 3. Segment *A*, non-distended; segment *B*, distended; segments *C* and *D*, overdistended; segment *E*, immediately after venesection. The second beat in segment *D* is probably a ventricular escape.

therefore not an artefact but an actual record of the pressure change within the ventricle at this time.

The diastolic dip is merely an exaggeration of the normal pressure drop found at this time in the pressure curve. It simply indicates that the ventricular aspiration is augmented so that the flow of blood into the heart follows the relaxation process less closely than usual. The depth of the dip is a rough index of the magnitude of the aspirating action of the relaxing ventricle. The greater size of the diastolic dip in the right ventricle is due to the fact that even in the non-distended heart the difference between the auricular and diastolic arterial pressure is much less than on the left side. This is also shown by the straddle of the pressure curve of the left ventricle being longer than the right. In other words, the right ventricle spends less time in isometric relaxation and more of its relaxation time in aiding filling than does the left. Filling therefore occurs in the right heart at an earlier stage in relaxation when the vigor of the process is greater than at later stages in relaxation. When the heart is distended

and overdistended the isometric relaxation period is further abbreviated, and this together with the augmentation of the vigor of relaxation makes the diastolic dip more prominent. The same explanation can be offered for the appearance of the diastolic dip in the left ventricle when it is distended.

The evidence here presented indicates that in addition to the passive filling of the ventricles by the blood returning to the heart, there is another process facilitating filling which depends on the relaxation of the ventricles and manifests itself as an aspirating action. The heart in this regard resembles an aspirating pump. The drop in pressure is created by the heart (pump); the energy for flow comes from the head of pressure in the reservoir.

The filling of the heart is therefore a twofold process; on the one hand, a passive one depending on the stored energy present in the capillary blood and the energy imparted to the blood by extracirculatory process such as respiration and muscular movement; on the other hand, the aspirating action of the relaxing ventricle which drops the pressure within its chamber despite the increase in its volume. In this regard the heart in filling its chamber acts in much the same way that inspiration acts in causing a flow of blood into the intrathoracic veins. The rapid inflow phase differs therefore from diastasis which occupies the rest of diastole in that the aspirating action of the ventricle is operating in the former period. A full discussion of this aspirating action has already been presented in a previous report (1930).

SUMMARY

In the course of these experiments a diastolic dip was seen, resembling the one described in the isolated turtle heart by one of us (L.N.K.), when the heart was distended and overdistended. It was always observed in the right ventricular pressure curve and occasionally in the left. Its size in the right ventricular curve was a direct function of the diastolic size of the heart. The diastolic dip is not an instrumental artefact but expresses the pressure changes in the ventricle at this time. The dip is an exaggeration of the normal drop in pressure present in the mammalian heart in the rapid filling phase and expresses an augmentation in the disparity between the rate of ventricular expansion and the rate of filling. An intraventricular pressure below atmospheric was never found in these experiments.

The diastolic dip indicates that ventricular relaxation plays a rôle in filling the mammalian heart by exerting an aspirating action during the rapid inflow phase.

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THE EXCRETION OF URINE IN THE DOG

I. The Urea and Creatinine Clearances on a Mixed Diet

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It has been observed by Goldring (1931) that the excretion of urea in man, as judged by the urea clearance test of Möller, McIntosh and Van Slyke (1928) may show under certain circumstances very marked deviations from the normal in spite of the facts that standard conditions are observed and that there is apparently no renal pathology. So striking are these deviations, in comparison with the uniform results usually obtained, that when Doctor Goldring described them to us we undertook an examination of the excretion of urea and other substances in the dog with the hope of securing an explanation of these anomalous phases of renal activity. Although this hope has not been fully realized, our experiments have yielded a number of very interesting results which will be described in this and succeeding papers.

The present communication is an examination of the applicability of the urea clearance test to the dog, with special reference to the determination of the augmentation limit, and a comparison of the urea clearance with the creatinine clearance.

Methods. The dogs used in these experiments were adult females all of which were thoroughly tame and accustomed to being handled and catheterized. We consider this an extremely important point for even in our best trained dogs, we find that the first period of observation on any one day may be anomalous, due presumably to psychic disturbance. At no time were any of our dogs traumatized, etherized or subjected to any maltreatment. We find that a definite routine must be observed in handling dogs under observation; during an experiment a dog is kept in a cage and removed only for short periods to collect urine or blood.

Urine was collected by catheterization, great care being used to empty the bladder at the end of each period. Blood was collected in the middle of each period by puncture of the jugular vein through the skin. Urea was determined in serum and urine by the gasometric method of Van Slyke (1927) and creatinine by Folin's method (1922) on the Folin-Wu filtrate. Creatinine dissolved in 100 cc. of water was administered with sterile pre-

TABLE 1

Urea and creatinine clearances on normal dogs maintained on mixed diet, corrected to square meters of surface area (S.A.). Experiment 40 on the same dog as 44, and experiments 49 and 70 on the same dog as 73

EXPERIMENT NUMBER	S.A.	V URINE VOLUME	UREA		UREA CLEARANCE		CREATININE		CREATININE CLEARANCE	UREA CREATININE RATIO
			Serum	Urine	$\frac{UV}{S}/S.A.$ maximum	$\frac{U\sqrt{V}}{S}/S.A.$ standard	Serum	Urine	$\frac{UV}{S}/S.A.$	
	square meters	*cc. per minute*	*mgm. per 100 cc.*	*mgm. per 100 cc.*			*mgm. per 100 cc.*	*mgm. per 100 cc.*		
9	0.62	0.137	27.0	4,460	36.5	98.3				
		0.237	31.2	4,600	56.6	116.0	7.9	2,015	98.3	0.576
		0.280	34.8	4,305	56.2	106.0	11.9	2,690	102.0	0.550
		1.15	31.8	1,159	67.7		9.2	700	141.8	0.479
11	0.56	1.53	36.2	794	59.7		20.5	689	91.8	0.650
		0.417	35.3	2,600	55.0		11.6	2,070	132.0	0.417
		0.492	31.6	2,010	56.2		6.5	1,034	138.2	0.407
		0.936	33.7	1,177	58.5		4.4	394	149.0	0.394
12	0.78	0.183	36.7	6,420	41.0	95.0				
		0.168	36.7	6,490	38.0	91.4	14.9	4,480	64.9	0.585
58	0.76	0.375	20.0	2,375	56.0	92.2	24.4	5,378	108.0	0.518
		0.345	19.5	2,800	65.4	111.0	23.4	6,260	120.2	0.544
		0.340	20.9	3,120	66.4	115.0	22.4	5,970	119.0	0.558
		0.375	20.4	3,365	81.4	133.0	19.5	5,762	143.0	0.569
		0.328	21.5	3,045	61.0	106.8	19.5	4,595	98.1	0.622
49	0.77	0.259	38.4	5,142	45.0	87.9				
		0.333	38.4	3,897	44.7	76.1	36.4	6,720	79.5	0.562
		0.300	38.2	4,280	43.7	80.0	34.7	7,460	83.8	0.522
		0.259	37.2	4,940	44.3	89.2	32.6	7,460	77.0	0.585
70	0.77	2.95	37.2	472	48.5					
		5.08	35.1	261	49.9					
		4.82	33.1	257	48.3					
		2.13	32.1	491	42.3					
		0.90	32.1	988	36.0					
		0.238	31.5	2,504	25.0	51.2				
73	0.77	0.644	30.4	1,591	43.8					
		1.87	30.4	490	39.3					
		3.91	29.5	242	41.7					
		1.22	29.0	716	39.2					

TABLE 1—*Concluded*

EXPERIMENT NUMBER	S.A.	V URINE VOLUME	UREA		UREA CLEARANCE		CREATININE		CREATININE CLEARANCE	UREA:CREATININE RATIO
			Serum	Urine	$\frac{UV}{S}$/S.A. maximum	$\frac{U\sqrt{V}}{S}$/S.A. standard	Serum	Urine	$\frac{UV}{S}$/S.A.	
	square meters	*cc. per minute*	*mgm. per 100 cc.*	*mgm. per 100 cc.*			*mgm. per 100 cc.*	*mgm. per 100 cc.*		
61	0.73	0.656	16.8	1,184	63.7					
		3.75	16.7	211	65.4					
		5.00	16.4	158	66.1					
		5.80	17.1	141	65.5					
69	0.62	4.32	17.9	144	55.7					
		4.80	17.9	128	55.3					
		0.720	17.8	909	57.8					
		0.172	17.4	2,268	36.3	87.1				
40	0.79	0.400	56.3	8,175	73.4 (Urea administered)					
		2.70	61.6	1,640	91.0					
		5.02	66.3	835	80.5					
44	0.79	2.50	76.9	1,650	67.9 (Urea administered)					
		3.8	69.8	1,085	74.7					
		2.6	65.3	1,292	65.0					

cautions by subcutaneous injection in order to obtain blood levels of this substance sufficiently high to permit accurate analysis. The blood was defibrinated and centrifuged immediately after it was drawn, and tungstate filtrates were made from the serum at once. Creatinine analyses were made on these and on diluted urines at the conclusion of the experiment by comparison with a creatinine standard (Folin, 1922). The interval of color formation was timed exactly to fall between 12 and 15 minutes and the colorimetric readings were made by one individual (A.B.) who has had extensive experience with this analysis. We consider the urea values to be correct to less than 1 per cent and the creatinine values to less than 2 per cent, as shown by control experiments on the recovery of these substances after the addition of known amounts to blood and urine.

The diet upon which the dogs are maintained, we discovered after a considerable number of experiments, is a very important factor in the urea clearance test even when this test is made 18 hours after the last meal. The data presented in this paper were obtained on dogs which were fed a common mixed diet of cooked meat scraps, chicken, bone, bread, potatoes,

etc. All experiments reported here were performed 18 hours after the last meal. These data are therefore comparable with those recently reported by Ralli, Brown and Pariente (1931) and differ from the latter principally in covering a wider range in the rate of urine excretion, and by the simultaneous determination of the creatinine clearance.

Discussion. *The standard and maximal clearance.* Table 1 gives the data from 43 observations on 8 dogs. These data (dots) are also given graphically in figure 1, together with data (circles) previously published by Ralli, Brown and Pariente (1931). Our data are principally concerned with relative high urine flows, while most of those of Ralli, Brown and Pariente are below 0.3 cc. per minute. It is necessary to take all these

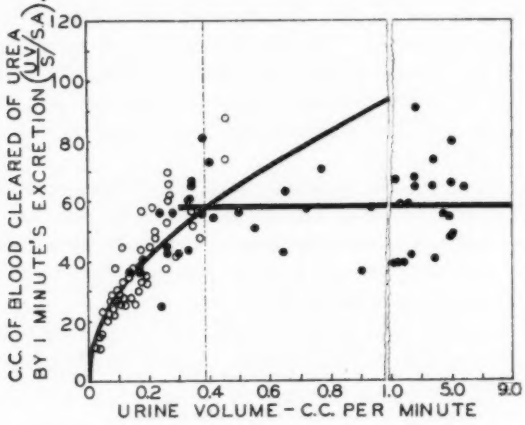

Fig. 1. Urea clearance in relation to urine volume in dogs fed a mixed diet. The curved line is calculated according to $\frac{UV}{S}\Big/S.A. = 92.8$ and intersects the horizontal line $\left(\frac{UV}{S}\Big/S.A. = 58.0\right)$ at the augmentation limit.

data into consideration to determine the augmentation limit. Ralli, Brown and Pariente have shown for individual dogs, or for various dogs when compared on the basis of surface area, or better yet, of kidney weight, that the excretion of urea conforms to the relation $\frac{U}{B}\sqrt{V/S.A.} = 99.5 \pm 21.9$ (where U and B are urea concentrations in the urine and blood respectively, V is the urine volume in cubic centimeter per minute and $S.A.$ is the surface area of the dog in square meters as calculated by the formula of Cowgill and Drabkin (1927)).

Our own data show that for rates of urine flow above 0.4 cc. per minute,

the excretion of urea is not influenced in individual dogs by variations in urine volume (expts. 11, 70, 73, 69, and 40) beyond statistically significant limits. There is more variation between different dogs than in individuals, but the mean maximal clearance for all data is 58.0 ±11.9. Austin, Stillman and Van Slyke (1921), examined two dogs under similar conditions. In all but one observation the urine volume was above the augmentation limit defined below; if typical surface areas are assigned to these dogs, the average urea clearances would be 63 and 70 per square meter of body surface.

We have included in table 1 and figure 1 a number of experiments in which creatinine was administered subcutaneously (500 mgm. per kgm.) and the creatinine clearance was determined at the same time as the urea clearance. It is significant that this administered creatinine does not affect the rate of urea excretion. In experiment 11 the maximum clearance averaged 57.3 as compared with our mean of 58.0. In experiments 9, 12, 58 and 49, in which creatinine was also administered, the standard urea clearance was 97.7 as compared with the mean of 99.5 observed by Ralli, Brown and Pariente.

The possibility that the administration of creatinine might influence the excretion of urea has been fully considered by us, and further observations on this point will be reported later. It may be noted now, however, that the urea clearance was the same in one dog reported here with and without creatinine (experiments 49 and 70).

In view of the uniformity of these results we have included all the above data, as well as those of Ralli, Brown and Pariente in the determination of the augmentation limit.

Calculation to the second approximation yields:

$$\text{Standard clearance, } \frac{U}{S}\sqrt{V}/S.A. = 92.8 \pm 19.4 \text{ (66 observations)}$$

$$\text{Maximal clearance, } \frac{UV}{S}\Big/S.A. = 58.0 \pm 11.9 \text{ (29 observations)}$$

$$\text{Average augmentation limit} = 0.38 \text{ cc. per minute.}$$

We find that both groups of data are statistically true when analysed by Dunn's (1917) method. In order to express the standard or maximal urea clearance in percentage of the normal (as Möller, McIntosh and Van Slyke have done) it is only necessary to multiply the former by 1.08 and the latter by 1.73. We do not recommend the adoption of these values, however, because as we will show in a subsequent paper, the nature of the maintenance diet has a profound effect upon the urea clearance test, and this factor must be controlled in any experiments upon renal function in the dog.

The creatinine clearance. We find that the urea clearance is consist-

ently about half of the creatinine clearance (actually 53 per cent in the average of the data reported here). A similar discrepancy between the excretion of creatinine and of urea has been observed by Rehberg (1926) in man and by MacKay and Cockrill (1930) in rabbits. Rehberg has interpreted his results to indicate that urea diffuses passively from the tubular urine back into the blood, but we are unable to accept this explanation entirely in the case of the dog because we find that the amount of urea which would be presumably reabsorbed bears no relationship to the concentration of urea in the urine. It may be recalled in this connection that Austin, Stillman and Van Slyke (1921) were led to the use of $\sqrt{V}$ in defining the urea excretion in man at low rates of urine flow because they found no correlation between the rate of urea excretion and the urea concentration in the urine.

It should be noted that the above data on the excretion of creatinine have no bearing on the normal excretion of this substance, for Behre and Benedict (1922) have shown that there is no significant quantity of creatine present in normal dog blood the creatinine excreted under normal conditions is formed by the kidney, probably from some precursor in the blood (cf. also Gaebler and Keltch, 1928, and Gaebler, 1930).

SUMMARY

The excretion of urea by the normal, unanesthetized dog fed upon a mixed diet can be described with statistical correctness by the maximal and standard urea clearance equations of Möller, McIntosh and Van Slyke.

The augmentation limit is about 0.4 cc. per minute of urine flow, the maximal clearance is 58.0 and the standard clearance is 92.8 per square meter body surface.

The urea clearance, either maximal or standard, averages only 53 per cent of the simultaneous creatinine clearance when creatinine is administered to raise the blood level.

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STUDIES ON THE URINARY EXCRETION OF OESTRIN, WITH ESPECIAL REFERENCE TO THE EFFECT OF THE LUTEINIZING HORMONE AND PROGESTIN

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During the course of the last year and a half various experiments upon rabbits in this laboratory have involved the injection of large amounts of oestrin (theelin, folliculin, female sex hormone, etc.). We have been continually puzzled by the fact that, although physiological results such as the growth of the uterus and breasts have been evident, the Allen-Doisy rat test upon the urines of the animals injected have never revealed more than traces of this hormone. Moreover, analyses of the urines of women who have been receiving large amounts of oestrin in various forms, both orally and subcutaneously, have yielded similar results, with only occasional exceptions in which definite excretion could be demonstrated during treatment. Further, we gave a normal man one thousand rat units of oestrin orally in one dose without being able to recover any in the urine during the following four days. It was not until studies were made with the luteinizing hormone and progestin in connection with the excretion of oestrin that any clue to this situation was discovered.

Quantitative estimation of urinary oestrin. It is inconvenient and often impossible to get 24-hour urine specimens on clinic and hospital patients. Inasmuch as the concentrations of different specimens vary greatly, it is also impossible to get a quantitative idea of the excretion of any substance unless one can know what part of the 24-hour output a given specimen represents. It has been shown by Folin (1) that urinary creatinine is entirely of endogenous origin and that the amount excreted in 24 hours is a constant and in exact proportion to the weight of the individual. We therefore, in testing for oestrin, use creatinine as a gauge of the concentration in a given specimen, injecting into spayed rats the volume equivalent to a certain number of milligrams. From this and the weight of the patient the 24-hour output of oestrin could be calculated, but, since comparable results are all that are required, this is not necessary. We have demonstrated with urines from pregnant women that the 24-hour output of

oestrin of a given individual is the same (over a period of several days) whether the urine is very dilute or very concentrated, and further, that the excretion of oestrin does not vary at different times of the day. Results are expressed, therefore, in terms of milligrams of creatinine equivalent to one rat unit of oestrin. The urines are first made acid to litmus paper with dilute sulphuric acid. Creatinine is then measured by the simple Folin colorimetric method (2). The urinary oestrin is determined by the Allen-Doisy method (3). Two rats are injected for each test, but since it is almost always necessary to repeat a number of times in order to trace down the exact quantity of urine that will give oestrous smears, six or more rats are usually used on each urine specimen. In rabbit experiments the same methods have been employed. The animals have been kept in metabolism cages and the urines collected daily, so that the 24-hour excretion of oestrin could also be calculated.

Oestrin in the blood and urine of pregnant women receiving the luteinizing hormone. On the theoretical assumption that the bleeding of threatened miscarriage in early pregnancy might be the result of a corpus luteum deficiency, a number of such cases at the Boston Lying-in Hospital are being treated with an extract of the urine of pregnant women which causes luteinization in the ovaries of experimental animals (prepared according to the method of Evans (4) and generously supplied to us by Parke, Davis & Company). Through the coöperation of Dr. John Rock and the Boston Lying-in Hospital, we have been able to follow the blood and urine of eight of these women. It was discovered that in all eight the oestrin of both blood and urine was definitely decreased after four or five days of treatment. As a control we have followed four patients during a week of routine hospital treatment for miscarriage before administration of the luteinizing hormone. In these four cases no change in the level of oestrin was found. Of the eight women receiving luteinizing hormone, six stopped flowing and vomiting temporarily and three of these have had no recurrence of symptoms. However, since routine hospital care alone often cures these cases, the clinical results can be considered no more than hopeful.

Rabbit experiments. These findings on pregnant women led us to investigate the effect of the luteinizing hormone and of progestin upon the excretion of oestrin in rabbits. Both normal and spayed does were used, all being first tested for excretion of oestrin after receiving theelin[1] alone. A control 24-hour specimen of urine was first collected, acidified, the volume then measured and the creatinine determination made. In all the experiments to be reported the same lot of theelin was used and 18 cc. were injected subcutaneously in one dose. As tested on our rats this amount is

[1] In this work we are much indebted to Dr. E. A. Sharp and the Parke, Davis Company for supplying theelin, anterior pituitary extracts and progestin.

TABLE 1

Rabbit 184—normal

EXPERIMENT I—EXCRETION OF THEELIN ALONE						
Specimen	Excretion of Theelin—gauged by cc. ⇔ 1 mgm.C.*					R.U. Theelin excreted Estimated by 24° volume
	1 mgm.C.	2 mgm.C.	5 mgm.C.	10 mgm.C.	20 mgm.C.	
No. 1. 24° 5/5/31				−	−	0
5/5/31	18 cc. Theelin (600–700 R.U.) subcutaneously					
No. 2. 24° 5/6/31		−	+	+	++	16
No. 3. 24° 5/7/31		−	±	+	++	20
No. 4. 24° 5/8/31				−	−	0
Total R.U. excreted over 4-day period.....						36

EXPERIMENT II—EXCRETION OF THEELIN AFTER LUTEINIZING HORMONE						
Specimen	Excretion of Theelin—gauged by cc. ⇔ 1 mgm.C.					R.U. Theelin excreted Estimated by 24° volume
	1 mgm.C.	2 mgm.C.	5 mgm.C.	10 mgm.C.	20 mgm.C.	
5/ 9/31	10 cc. Luteinizing Hormone subcutaneously (1 cc. ⇔ 50 + R.U.)					
No. 1. 24° 5/10/31				−	+	4
5/10/31	18 cc. Theelin (600–700 R.U.) + 10 cc. Luteinizing Hormone s.c.					
5/11/31	10 cc. Luteinizing Hormone s.c.					
No. 2. 24° 5/11/31	±	+	++	++		180
No. 3. 24° 5/12/31	−	+	+	++		96
No. 4. 24° 5/13/31		−	+	+	++	20
No. 5. 24° 5/14/31				−	+	10
No. 6. 24° 5/15/31				−	−	0
Total R.U. excreted over 6-day period.....						310

* C. = Creatinine.

TABLE 2

Rabbit 194—normal

EXPERIMENT I—EXCRETION OF THEELIN ALONE						
Specimen	Theelin in urine—gauged by cc. ⇔ 1 mgm.C.*					R.U. Theelin excreted Estimated by 24° volume
	0.5 mgm.C.	1 mgm.C.	2 mgm.C.	5 mgm.C.	10 mgm.C.	
No. 1. 24° 5/16/31					−	0
5/16/31	18 cc. Theelin (600–700 R.U.) subcutaneously					
No. 2. 24° 5/17/31				−	+	6
No. 3. 24° 5/18/31			−	+	++	25
No. 4. 24° 5/19/31					−	0
Total R.U. excreted over 4-day period.....						31

EXPERIMENT II—THEELIN EXCRETION AFTER PROGESTIN						
Specimen	Theelin in urine—gauged by cc. ⇔ 1 mgm.C.					R.U. Theelin excreted Estimated by 24° volume
	0.5 mgm.C.	1 mgm.C.	2 mgm.C.	5 mgm.C.	10 mgm.C.	
5/27/31	1 cc. Progestin subcutaneously					
No. 1. 24° 5/28/31					−	0
5/28/31	18 cc. Theelin (600–700 R.U.) + 1 cc. Progestin s.c.					
5/29/31	1 cc. Progestin s.c.					
No. 2. 24° 5/29/31	±	+	++	++	+++	375
No. 3. 24° 5/30/31	−	+	+	++		155
No. 4. 24° 5/31/31					−	0
Total R.U. excreted over 4-day period.....						530

* C. = Creatinine.

equal to between 600 and 700 rat units of oestrin. The 24-hour urines were collected daily after the injections until a specimen was acquired

which gave a negative rat test with the amount equivalent to 20 mgm. of creatinine. The content of oestrin in a given specimen was estimated by dividing the volume by the smallest number of cubic centimeters which gave a positive rat test, the sum of these values being considered the total excretion of oestrin. Six experiments were run in which theelin alone was given, three of the does being normal and the other three spayed. Of the 600 to 700 rat units injected, only between 22 and 67 units were recovered in the urines.

We next tried the effect of luteinizing hormone upon two of these normal does. The excretion of oestrin was ten times greater than when theelin alone was given. Table 1 illustrates the procedure followed and gives the results of one of these experiments.

TABLE 3

The effect of the luteinizing hormone and of progestin upon the excretion of theelin injected into rabbits

R.U. excreted in 24° volumes of urine

24° URINES	AFTER THEELIN ALONE 600–700 R.U.				AFTER THEELIN 600–700 R.U. AND LUTEINIZING HORMONE			AFTER THEELIN 600–700 R.U. AND PROGESTIN		
	Normal		Spayed		Normal	Spayed		Normal	Spayed	
	No. 184	No. 194	No. 51	No. 180	No. 184	No. 51	No. 180	No. 194	No. 51	No. 180
Before injection	0	0	0	0	4	0	0	0	0	0
1st day after	16	6	50	9	180	60	10	375	270	480
2nd day after	20	25	17	13	96	16	16	155	70	120
3rd day after	0	0	0	0	20	0	0	0	0	0
4th day after					10					
5th day after					0					
Total R.U. theelin excreted	36	31	67	22	310	76	26	530	340	600

In order to discover whether this increased excretion was the direct result of the luteinizing hormone or was due to its luteinizing effect upon the ovaries, we repeated this experiment on two spayed rabbits. These animals showed the same low urinary output of oestrin as after theelin alone.

Progestin was next given with theelin. The lipoidal extract of Corner and Allen (5) was used. The experiments were run on the two spayed does which had shown no response to the luteinizing hormone and upon one normal doe rabbit. Table 2 illustrates the procedure followed and gives the results in one animal. With progestin the recovery of injected oestrin in both spayed and normal rabbits is even more marked than with the luteinizing hormone in normal does.

As shown by the summary in table 3, progestin is necessary for the excretion of oestrin in rabbits, and also the increased excretion by normal rabbits after the luteinizing hormone must be due to corpus luteum formation. The results on normal doe 184 (see table 1) when it received luteinizing hormone are particularly interesting in connection with this last. This rabbit had one 10-cc. injection of the luteinizing hormone on the day before theelin was given. There was a slight excretion of oestrin before the injection of theelin; the 24-hour urines gave positive tests for oestrin two days longer than did those of rabbits getting progestin; the total excretion was less than after progestin. All three of these observations are in keeping with the conclusion that the effect of the hormone from pregnant urines upon the excretion of oestrin is through its luteinizing activity upon the ovaries.

This finding of the rôle of progestion in promoting the excretion of oestrin explains why we were unable to recover in the urine of rabbits the oestrin injected, in spite of the absorption evident from the physiological effects upon the animals.

Urinary excretion following the administration of oestrin to amenorrhea and sterility patients. The above finding also offers a clue to the failure to detect any appreciable urinary excretion in the majority of human cases who have been taking oestrin in very large amounts. In the Out Patient Clinic at the Free Hospital for Women, various preparations of oestrin have been administered to sterility and amenorrhea patients (orally in the form of progynon[2] and a crude but potent extract of pregnant urine; subcutaneously in the form of theelin). The clinical results have not been encouraging. On 9 of these patients we have tested the urines for oestrin from one to four times during treatment, analyzing 20 specimens in all. Of these, only 3 specimens gave oestrous smears in rats when tested in amounts equivalent to 10 or 20 mgm. of creatinine. One woman who had been taking 9 tablets of progynon daily for a month with the hope of alleviating complete amenorrhea showed no oestrin in any of the three specimens tested during this period. After treatment with the luteinizing hormone and progynon, however, oestrin was demonstrable in the urine. Another woman receiving 9 tablets of progynon daily showed excretion (i.e., $^{++}$ test for oestrin with the amount of urine equivalent to 10 mgm. C) at the time when the ovary undoubtedly contained an active corpus luteum, that is, 5 days before menstruation. This may, therefore, be of diagnostic value. If an amenorrhea case receiving large doses of oestrin shows no excretion at any time during the month, it may be assumed that no ovulation, with subsequent corpus luteum formation, has occurred and that there is either inadequate anterior pituitary activity

[2] Prepared by Schering-Kahlbaum A. G. Berlin and kindly supplied to us by the Schering Corporation of New York.

or deficient ovarian response. The fact that 7 out of the 9 sterility and amenorrhea cases followed showed no excretion after treatment is perhaps significant. Oestrin alone would not be expected to alleviate this condition unless it activated the hypophysis, and there is as yet no conclusive evidence for this.

Discussion. Zondek (6), in studying the excretion of oestrin during the normal human cycle, reports that only very small amounts are ever present in the urine, but that in the premenstrual period three times as much is found as in the intermenstrual period. This is interesting in the light of our finding of the effect of progestin upon the excretion of oestrin, for the premenstrual period is presumably the time when the corpus luteum is most active. It has been demonstrated in monkeys (7) that menstruation following the administration of oestrin will not occur until the oestrin injections have been decreased or discontinued. This would indicate that although oestrin is needed to prepare the endometrium for menstruation, the anterior pituitary hormone responsible for bleeding (8) cannot act until the level of oestrin is lowered. It may be, therefore, that progestin plays an important rôle in causing the excretion of oestrin during the premenstrual period.

It has been found in animals (9) that early pregnancy may be interrupted by the injection of oestrin. Frank (10) has suggested that there may be a lowered renal threshold for oestrin during gestation. Our results indicate that the active corpus luteum of early pregnancy may, as a protective mechanism, have just this effect. Moreover, in the cases of threatened miscarriage reported above there was some indication of abnormally large amounts of oestrin in both the bloods and urines. We have not as yet sufficient controls to warrant any definite conclusions in this respect. It seems logical to assume, however, that during the first months of pregnancy progestin has among its other important functions that of keeping the oestrin below dangerously high levels by promoting its excretion. The increasing amounts found in the urine as gestation advances cannot be explained on this basis, since the corpus luteum is known to regress during the last months. The high levels of oestrin in the blood during later pregnancy may be sufficient to account for its excretion without any lowering of renal threshold, and the more developed fetus is apparently unharmed by large amounts of this hormone.

The ability of progestin to increase oestrin excretion may be of physiological importance, therefore, both in the menstrual cycle and during gestation, and abnormal conditions accountable to excessive oestrin may have as a causative factor insufficient corpus luteum activity.

SUMMARY

1. A lowering of the level of oestrin has been demonstrated in pregnant patients receiving the luteinizing hormone.

2. Experiments on normal and spayed doe rabbits receiving theelin alone and theelin together with the luteinizing hormone or progestin have led to the conclusion that progestin causes the excretion of oestrin.

3. This finding may explain the failure to recover oestrin in the urines of the majority of human amenorrhea and sterility cases receiving large amounts of this hormone.

4. The possible importance of the corpus luteum in promoting the excretion of oestrin during the menstrual cycle and early pregnancy is discussed.

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THE EFFECT OF THE VAGUS ON THE DIASTOLIC SIZE OF THE HEART AND ITS BEARING ON THE QUESTION OF CARDIAC TONUS

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Meek (1927) has critically reviewed the literature on cardiac tonus. It appears that cardiac tonus, as he defines it, does not exist in those forms whose heart musculature consists exclusively of cardiac muscle. The present paper contains further evidence that the vagus nerve has no direct effect upon the diastolic size and hence exerts no "tonogenic" influence on the heart.

METHOD. These experiments were done on a series of eight barbitalized dogs. The trachea was cannulated and the right vagus exposed and cut. An x-ray picture of the heart was obtained using a constant target film distance of one meter. A second picture was made during vagus stimulation to establish the functional reliability of this nerve. The intrapulmonic pressure was then raised to 120 mm. or more of water by blowing into the trachea and clamping it. A control series of pictures was obtained 10, 30 and 50 seconds after clamping. The tracheal cannula was unclamped and the animal allowed several minutes to recover. The experimental series, as in the control, consisted of pictures taken 10, 30 and 50 seconds after the intrapulmonic pressure had been raised, but the vagus was stimulated simultaneously with exposure of the second film. That is, in the experimental series the picture made 30 seconds after raising the intrapulmonic pressure showed the effect of both the raised pressure and vagus stimulation. The animal was again allowed to recover. The vagus was stimulated to test its continued efficacy and the entire experiment repeated.

RESULTS. The area of the cardiac silhouette was measured in each case. Table 1 summarizes the results obtained in one dog in detail and the averages of the eight. It is significant that the size of the heart does not remain constant after it has been emptied of blood. There is a successive increase in size so that it is of paramount importance to take the exposures at definite time intervals after raising the intrapulmonic pressure. It will also be noted that in no case were results obtained during vagus stimulation significantly different from those obtained in the control series where the

vagus was not stimulated. That is, the stimulation of the vagus was without effect under the controlled conditions of the experiment, the most important being venous pressure, which, we believe, was amply controlled by raising the intrapulmonic (hence intrathoracic) pressure above the level of the venous pressure in the large vessels entering the heart.

Discussion. There is no definitely positive explanation of the progressive increase in the size of the heart. Possibly it is the response to an anemia and asphyxia resulting from the lowered blood pressure caused by the lack of filling of the left heart which Johnson and Luckhardt (1928)

TABLE 1

	Representative animal		Average of eight
	Cardiac area	Changes in terms of normal	Changes in terms of normal
	sq. cm.	*per cent*	*per cent*
Normal cardiac area	38.70	100	100
Area during vagus stimulation	46.12	119	114
Raised intrapulmonic pressure:			
Control series I:			
End of 10 seconds	27.74	72	78.5
End of 30 seconds	30.96	80	81.4
End of 50 seconds	33.22	86	87.8
Experimental series I:			
End of 10 seconds	28.38	73	79.5
End of 30 seconds. Vagus stimulated	30.64	79	81.3
End of 50 seconds	31.61	82	84.6
Control series II:			
End of 10 seconds	28.06	73	75.3
End of 30 seconds	29.99	77	79.2
End of 50 seconds	31.61	82	84.7
Experimental series II:			
End of 10 seconds	27.74	72	75.6
End of 30 seconds. Vagus stimulated	30.32	78	80.0
End of 50 seconds	30.96	80	82.4

have shown to follow a rise in the intrapulmonic pressure. The easily demonstrated fact that anoxemia causes a progressive increase in the diastolic size of the heart varying, within limits, with the time supports this view.

A second possibility is that an increased intrapulmonic pressure "markedly slows the heart for a prolonged period by stimulating the center directly" which effect is prevented by double vagotomy (Luckhardt and Johnson, 1928). A decreased heart rate is recognized as allowing greater cardiac relaxation. This does not well explain the process in our experi-

ments, however, because the dilatation becomes successively greater and it is hard to see how a nervous effect could be so cumulative. In view of our negative results in this experiment it seems impossible that there is a reflex effect on "cardiac tone" through the intact left vagus.

A third possibility is seepage during raised intrapulmonic pressure. It is impossible to assert that no blood entered the heart because it is hard to understand why a heart would dilate without some intracardiac force distending it. In fact if it were not for this factor any tendency towards inhibition which the vagus might have might be masked because there would be nothing to demonstrate that the walls of the heart were less resistant to distending tendencies.

A fourth possibility is that the pulmonary venous return is obstructed before the vena cava return leading to an acute passive congestion of the lungs. This, especially in case of seepage, would tend towards a right heart enlargement so that the enlarging cardiac silhouette would result primarily from right cardiac enlargement.

Meek (1927) lists as factors which must be controlled in studying cardiac tone: "the temperature, the carbon dioxide and oxygen content, the influence of nerves, the distending pressures and the chemical composition of the nutrient media." We did not, as he suggested, keep all but one of these constant. We did, however, control them, for in the control series we measured the effect of all the factors except the influence of nerves, and then during the experimental series we introduced the nervous factor already knowing the combined effect of the other factors. As a check on the presence of the nerve influence we demonstrated in every case that the vagus was functional before and after each experiment.

To test the possibility of the anemia or asphyxia suddenly decreasing the irritability or conductivity of the vagus, in several instances we stimulated it immediately upon release, after a period of one minute's duration, of the raised intrapulmonic pressure. In these cases a typical vagus effect was demonstrated. While this does not absolutely settle the question, it is reasonable to assume that the recovery from a depression would not be so much more rapid than the development of the condition that the vagus could be considered paralyzed at the time of stimulation during raised intrapulmonic pressure. On the other hand anemia may cause an acutely refractory state of the cardiac muscle itself to any vagus effect.

In our experiments venous pressure was rigidly controlled because the raised intrapulmonic pressure hindered blood from entering the heart. When the venous pressure was thus controlled vagus stimulation had no effect upon the diastolic size of the heart. These findings suggest that the vagus has no "tonogenic" effect on the heart. In addition, under the cardiac rates of our experiments the heart during raised intrapulmonic pressure relaxed as completely during diastolic as it did when vagus inhibition was

superimposed. That is, changes in the rate *per se* did not cause an increase in cardiac size. This would seem to indicate that more complete relaxation in uncontrolled experiments, allowed by increasing the relaxation time by vagus stimulation, is relatively less important in determining diastolic size than is the distending action of a venous pressure allowed to act over a greater time. It would seem, therefore, that any effect which the vagus may have on cardiac dilatation is not directly through its effect on a tone mechanism, if, indeed, there is such a thing as true cardiac tonus at all.

CONCLUSION

Whether or not there is such a thing as cardiac tonus, the vagus nerve does not, within the limits of this experimental method, function in the mechanism.

We wish to thank Mr. J. C. Hall for valuable technical assistance during the entire course of these experiments.

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STUDIES IN THE PHYSIOLOGY OF VITAMINS

XVI. The Effect of Exercise on the Time Required for the Development of the Anorexia Characteristic of Lack of Undifferentiated Vitamin B[1]

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Previous work in this laboratory (Cowgill and Klotz, 1927) indicates that the amount of the vitamin B complex required for maintenance of the urge to eat by pigeons, rats and dogs may be estimated quite accurately by an expression of the following type:

(1) $$\text{Vitamin} = K.\ \text{Weight}^{5/3}$$

where the value of K is peculiar to the species. If this is written differently as

(2) $$\text{Vitamin} = K.\ \text{Weight}.\ \text{Weight}^{2/3}$$

it is seen that the vitamin requirement may be regarded as a function of the product of the mass of tissue (body weight) and the metabolism of that mass (two-thirds power of the weight). Students of metabolism are aware that the basal metabolic rate at least is a close function of the body surface area which can be estimated in most cases with reasonable accuracy by a formula of the Meeh-Rubner (Rubner, 1883) type where body surface area is given as a linear function of Weight $^{2/3}$, namely

(3) $$\text{Surface Area} = K.\ \text{Weight}^{2/3}$$

It is evident from the work of one of us (Cowgill, 1928) that under uniform conditions of feeding and cage life, the total metabolism also is a close func-

[1] The physiological properties of what was formerly called *vitamin B* have since been shown to be due to at least two substances which have been designated vitamins *B* and *G* by some investigators, and B_1 and B_2 by others. The terms *undifferentiated vitamin B* and *vitamin B complex* used in this paper refer to the mixture of these two substances present in the product used as a source of vitamin.

[2] Part of the data reported in this paper are contained in the thesis submitted jointly, April, 1930, by H. A. Rosenberg and J. Rogoff to the faculty of the Yale School of Medicine in partial fulfillment of the requirements for the degree of Doctor of Medicine.

tion of the two-thirds power of the body weight, and therefore, in a formula of type 2 above, the expression Weight $^{2/3}$ might mean *total* metabolism as well as *basal* metabolism. When expression 2 is rearranged as in 4,

$$\frac{\text{Vitamin}}{\text{Weight}} = K \cdot \text{Weight}^{2/3} \tag{4}$$

it is seen that the vitamin requirement per unit of weight is a linear function of the two-thirds power of that weight. In other words, the vitamin requirement per unit of tissue mass is proportional to the metabolism of that mass. If, under suitable experimental conditions it were possible to keep the individual's weight constant but to increase the metabolism markedly, then, according to expression 4 and the meaning being given to $W^{2/3}$, namely, total metabolism, the amount of vitamin needed by the animal should be found to be correspondingly greater. One way in which the total metabolism of an animal may be increased is by means of forced exercise with consequent ingestion of larger amounts of food. A study of the effect of exercise on the requirement for undifferentiated vitamin B, therefore, should yield data tending either to support or to disprove the interpretation given the quantitative studies from this laboratory mentioned above.

It is quite generally agreed that the animal body's capacity for storage of the antineuritic vitamin, which is the component of the B complex that plays the dominant rôle in maintaining the normal urge to partake of food (Cowgill, Rosenberg and Rogoff, 1931) is fairly limited, in contrast to its ability to store other dietary factors, the fat-soluble vitamin A substance, for example. This fact may be utilized in studies of the type reported in this paper in developing a criterion of the existence of a state of vitamin "B" deficiency.

When a standard experimental ration such as our *casein III* (Cowgill, 1923) is fed to dogs under conditions which insure that a pseudoanorexia due to adjustment of the animal's caloric intake to its energy demands (Cowgill, 1928) does not occur, the time required for development of the characteristic loss of the urge to eat due to lack of the B complex may or may not be quite constant depending upon the amount of the vitamin that was present in the diet previously available to the animal and stored in the tissues. When the dogs are given several large daily doses of vitamin-rich material in order to "saturate the tissue reservoir," so to speak, quite uniform results are obtained. In most cases about 21 days are required, with some animals exhibiting this anorexia as early as 18 or 19 days and others after as many as 30 or 33 days. If coprophagy occurs, the animal will not lose its urge to consume the experimental ration unless muzzled. It is reasonable to assume that when, under special experimental conditions, a marked shortening of this period occurs, there has been a more rapid

exhaustion of the vitamin store probably through consumption in metabolism or loss by excretion. The period required for this anorexia to develop, therefore, may be taken as evidence that the store of undifferentiated vitamin B has been depleted and requires replenishing.

This paper is a report of experiments on dogs in which the effect of forced exercise on the time required for depletion of the vitamin store, as evidenced by development of the characteristic anorexia, was determined and compared with the time required when the same animals were not given such exercise.

EXPERIMENTAL DETAILS. Five dogs were used in this study. Each animal was its own control with respect to the one variable, namely, exercise, studied in these experiments. Each dog was first given a large amount of undifferentiated vitamin B in the form of vitavose[3] in order to bring about a condition of "vitamin saturation." The animal was then allowed

TABLE 1

DOG	BODY WEIGHT WHEN		FOOD INTAKE DAILY WHEN		DURATION OF DAILY EXERCISE	TIME ON DEFICIENT DIET REQUIRED FOR DEVELOPMENT OF ANOREXIA WHEN		RATIO $\frac{y}{x}$
	Not exercised	Exercised	Not exercised	Exercised		Not exercised x	Exercised y	
	kilo	*kilo*	*grams*	*grams*	*minutes*	*days*	*days*	
C	7.8	7.8	118	128	45	21	13	0.61
I	11.4	10.5	135	200	90	21	11	0.52
F	12.0	12.0	183	193	45	18	10	0.55
G	12.0	12.3	180	250	90	18	8	0.44
D	13.7	13.8	150	200	90	24	14	0.58

to subsist on the vitamin B-deficient ration *casein III* (Cowgill, 1923) and the time required for development of the characteristic anorexia determined. When the urge to eat was lost, negative control experiments were performed in some cases by administering beef extract by stomach tube with subsequent negative effect on the anorexia; finally the urge to eat was restored by the administration of vitavose thus proving that the loss of appetite was really the characteristic one due to lack of undifferentiated vitamin B. After a rest period, the experiment was repeated but with the dog given forced exercise sufficient in degree to insure a daily intake of a significantly larger amount of the food in order to maintain the original body weight.

The exercise was afforded by means of a suitable treadmill operated by an electric motor at a speed kept constant as far as possible. The animals

[3] Obtained from E. R. Squibb and Sons, New York, N. Y.

were put through a preliminary period of training so as to be able to run from 45 to 90 minutes daily without interruption and exhaustion. Over another period of at least one week the animals were exercised and the extra amount of food required to maintain the original body weight under these conditions was determined. During this period the animals received liberal amounts of vitavose.

RESULTS. *In each case of increased exercise the period required for the development of anorexia characteristic of lack of undifferentiated vitamin B was decreased by from one-third to one-half of the time required when the dogs were not exercised.* When the loss of appetite had been corrected by administration of a sufficient amount of the vitamin, the animals maintained their appetite, even though they were still exercising. The data are summarized in table 1.

DISCUSSION. In view of the unanimity of results from these five dogs it was felt that further trials with this type of experiment were unnecessary. These findings certainly support the interpretation given the quantitative vitamin studies previously reported from this laboratory and discussed in the opening paragraphs of this paper.

Other investigators as well have expressed the opinion that the vitamin B requirement stands in some relation to metabolism. Randoin and Simonnet (1924) have suggested that $\frac{\text{vitamin B}}{\text{carbohydrate}} = \text{constant}$. Plimmer, Rosedale and Raymond (1927) came to the conclusion that $\frac{\text{vitamin B}}{\text{calories}} = \text{constant}$; various diets rich in protein, carbohydrate and fat, respectively, were fed and the *total caloric intake* rather than the particular foodstuff (carbohydrate, fat and protein) appeared to be the common denominator of interest. It is evident that the study described in this paper may be regarded as supporting all of these views as well as that of Cowgill and Klotz (1927) which served as the chief motivation of this research. The data here reported contribute nothing, however, to the question of the possible relation of undifferentiated vitamin B to the metabolism of any particular foodstuff.

The results of this research are also in accord with the clinical observations of McCarrison (1921) and Wood (1928) that human beriberi is brought on sooner by physical exertion. Further confirmation may be cited in the work of Yoshine (1922) who found that his exercised B-deficient rats died sooner than the non-exercised but B-deficient companions.

The results of this study do not agree however with those of Keith and Mitchell (1923), who exercised rats in revolving cages for from 30 to 40 hours per week, and reported that muscular exercise distinctly hastened the appearance of symptoms of vitamin A deficiency but failed to affect vitamin "B" metabolism. It is pertinent to point out that their tables

indicate the results of their experiments to have been highly variable, and that these authors did not give their exercised rats more food than the unexercised controls; furthermore, their criterion of physiological lack of undifferentiated vitamin B was failure of growth on the part of the young animals. The facts: (1) that their young rats were able to make some growth on the diet used and under the experimental conditions imposed, and (2) that only one out of a total of 28 (14 pairs) ever exhibited the symptoms of polyneuritis, indicate that the animals were receiving appreciable quantities of the antineuritic vitamin B. There is no evidence in the paper that coprophagy on the part of the experimental animals was prevented. In justice to these authors it should be pointed out that the importance of controlling the coprophagy factor was not generally appreciated at the time their experiments were performed. The data yielded by their studies with adult rats are perhaps more comparable with ours. Here it is noticed that every one of the 6 exercised animals lost considerable weight (from 46 to 134 grams) during the experiment, a fact indicating that the exercise was performed in considerable measure at the expense of body tissue instead of extra food. It may be questioned, therefore, whether the total metabolism was increased to any great extent by the exercise given in these experiments. The fact cited by Keith and Mitchell (1923) that their exercised rats as a rule did not eat more food than the unexercised companions in all probability means that, when they were not being exercised, these rats were resting more than the control animals, and as a result, their total metabolism approximated that of their companions. In the light of our own findings this would result in the detection of little or no difference in the vitamin requirements of the two groups of rats. Further experiments along this line should be performed and are included in the present research program of this laboratory.

It is of interest to record Blacklock's (1924) belief that *lack* of exercise hastens the onset of symptoms of vitamin B deficiency, this conclusion being based on the observations that beriberi is notoriously a disease found in places where there is a fixed diet and some degree of confinement, as in prisons, asylums and on shipboard. The fallacy of such reasoning is obvious.

CONCLUSION

Under conditions of forced exercise the period required for the development in dogs of the anorexia characteristic of lack of the vitamin B complex is markedly less than the time required when forced exercise is not given. These findings are considered to support the interpretation given the quantitative vitamin studies previously reported from this laboratory, that *the vitamin B requirement per unit of tissue mass is proportional to the metabolism of that mass.* These results also support the observations of

other investigators showing a relationship between vitamin B and metabolism.

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THE INFLUENCE OF PHYSICAL TRAINING ON THE BASAL METABOLIC RATE OF MAN[1]

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In a former study in this laboratory (Schneider, Clarke, and Ring, 1927) on the influence of a moderate amount of physical training on the basal metabolism of five men, it was observed that the metabolic rate per square meter of body surface declined in three but remained unchanged in two of the subjects. During a post-training period of several months, in which physical exercise was neglected, the rate of the three men in whom it had declined returned to that of the pre-training period.

In the following discussion we shall report on observations made on two groups of men. The first includes nine athletes, engaged in sports, on each of whom a series of basal metabolic determinations was made both while in and out of training. These men may be regarded as a naturally athletic group. The second includes three subjects selected because of their non-athletic habits who were under observation prior to, during, and following a period of six weeks of regular, but moderate, daily physical exercise.

The metabolic determinations were made with the Benedict Field Respiration Apparatus. This apparatus was carried to the bedside of the subject and, as a rule, the basal rate of metabolism was determined before he was permitted to stir or get out of bed. Each man was first familiarized with our purpose and trained to serve as a metabolism subject. Almost to a man we secured whole hearted and interested coöperation, so it is believed the data obtained are trustworthy. We have calculated the basal metabolic rate according to the height-weight formula of DuBois (1916) and have expressed it in per cent above or below the normal standards of Aub and DuBois (1917).

The athletes. Of the nine athletes who were under observation seven experienced a basal metabolic rate that was lower while in than when out of training; one showed no material change; while the last, the exceptional case, showed a rate that was clearly higher when in than when out of training.

[1] The expense of this investigation has been met by a grant from the Charles Himrod Denison Fund.

The personnel of the athletes includes two football men, two swimmers, two basket-ball players, one cross country runner, and two who participated in two or more sports, one of these going from soccer to swimming and the other from football into swimming and then into track. A comparison of the average rates in training and out of training for each sport is summarized in table 1.

The individual records of the metabolism of these men contain facts of interest. We have from 7 to 12 good observations on each man, except K. M. S. who was available only three times. An injury confined him to the hospital for a number of weeks.

TABLE 1

SPORT	SUBJECT	CALORIES PER SQUARE METER BODY SURFACE		BASAL METABOLIC RATE		
		Out of training	In training	Out of training	In training	Change as result of training
				per cent	*per cent*	*per cent*
Football	J.F.B.	40.30	38.46	+2.03	−2.63	−4.66
	K.M.S.	39.59	37.40	+0.23	−5.30	−5.53
	S.C.W.*	43.83	42.66	+6.90	+5.89	
Swimming	S.C.W.*		37.71		−7.99	−14.89
	M.R.P.	39.43	37.78	−0.19	−4.54	−4.35
	W.C.	41.05	40.68	+1.40	−0.77	−2.17
	E.F.H.†	45.19	40.39	+10.21	−1.53	−11.74
Basketball	H.C.W.	41.30	38.38	+0.74	−6.38	−7.12
	O.S.N.	42.33	38.34	+3.24	−6.48	−9.72
Cross-country	R.W.D.	40.36	45.06	−3.50	+9.89	+11.45

* In football, swimming and track. First set of observations made while training for football.

† In soccer and swimming.

The striking feature about J. F. B.'s record is that, while his in-training and out-of-training data do not range far from the normal of the DuBois standard, the in-training determinations were without exception below the normal; and the out-of-training results, with the exception of one obtained the first week after training, were all above the normal. Hence his basal respiratory exchange during training is lower by about 4.7 per cent than his out-of-training rate. His average while out of training was +2.03 per cent and his average during training was −2.63 per cent.

M. R. P. was never as completely out of training as J. F. B. and yet his

basal metabolism showed an average fall of 4.4 per cent during the period of training. During the period of training seven determinations were made over a period of 3 months in which the basal metabolic rate, with one exception of +1.40, ranged between −3.41 and − 7.94 per cent. In the four determinations of the post-training period three were slightly above the DuBois normal.

Subject E. F. H. went immediately from a season of soccer to one of swimming. His basal metabolic rate, beginning at +10.21 per cent, fell steadily in four successive weeks as follows: +7.25, +1.32, −0.84 and −5.98. During the remainder of the training period his rate fluctuated around −2.24 per cent. During the first month after the swimming season his basal metabolic rate rose slightly and was up to +2.02 per cent at the last determination, one month after training was discontinued.

Subject S. C. W. participated in three sports without breaking training, he went from football into swimming and then into the sprints of track. Starting with a metabolic rate of +6.90, it gradually fell to −10.32 and −11.39 per cent in the swimming season. Seven determinations were made during the training period. Of these only one was made during the track season and it was −9.12 per cent. The average rate for the last five determinations was −8.22. It appears, therefore, that the basal metabolic rate of S. C. W. fell, as a result of training, from +6.90 to −8.22 per cent.

O. S. N. is also a man in whom the basal metabolic rate clearly went down during a period of training. During the basketball season four determinations on him in a period of two months were as follows: −9.39, −7.05, −3.73, and −5.76 per cent. A week after training ceased the rate was still below normal, but all other out-of-training determinations were above normal. This subject therefore gave a training drop from +3.24 to an average of −6.48 per cent, or a change of −9.73 per cent.

One of our athletes, a cross-country runner, reacted in exactly the opposite manner from the above case. Subject R. W. D., when out of training, gave rates of −0.75 to −11.28, with an average of −3.50 per cent. His metabolic rates for three successive weeks toward the end of the training periods were +12.29, +5.76, and +11.68 per cent. Thus he shows an increase of 13.39 per cent, or from an average of −3.50 when out of training to +9.89 when in training.

Non-athletes. Our data on this group are limited to three subjects, but are in most respects more complete than those obtained from the athletes. These men superimposed, for a period of six weeks, at least an hour of vigorous physical exercise, such as handball, running or swimming, upon daily routine of laboratory work. Before participating in this study they had been thoroughly accustomed to serve as subjects for the determination of basal metabolism. A summary of the data for the training, post-train-

ing, as well as a few pre-training determinations of the basal metabolic rate is given in table 2.

The amount of exercise taken by the non-athletes resulted in improved appetite, feeling of well-being, and alertness. Each man also showed

TABLE 2

Basal metabolism of non athletes

A.O.F.			I.B.H.			G.W.H.		
Date	Calories per square meter per hour	Basal metabolic rate	Date	Calories per square meter per hour	Basal metabolic rate	Date	Calories per square meter per hour	Basal metabolic rate
			Pre-training					
1930		*per cent*	*1930*		*per cent*	*1930*		*per cent*
Jan. 18	36.31	−8.00	Jan. 5	38.85	−1.65	Jan. 16	36.11	−8.70
Jan. 22	35.32	−10.50	Jan. 10	38.23	−3.21	Jan. 24	35.44	−10.40
Jan. 24	36.54	−7.30	Jan. 12	37.94	−3.95	Jan. 25	37.71	−4.50
			Jan. 16	38.77	−1.81			
			Training					
Jan. 30	31.98	−19.00	Jan. 30	42.85	+8.48	Jan. 31	36.77	−6.90
Jan. 31	33.65	−14.81	Jan. 31	43.47	+10.04	Feb. 4	37.55	−4.90
Feb. 3	35.07	−11.21	Feb. 3	42.22	+7.00	Feb. 7	38.89	−1.55
Feb. 5	36.02	−8.81	Feb. 5	44.64	+13.00	Feb. 25	37.85	−4.19
Feb. 7	35.06	−11.24	Feb. 12	41.71	+5.60	Mar. 4	37.44	−5.23
Feb. 12	34.24	−13.32	Feb. 17	41.03	+3.86	Mar. 7	39.21	−0.74
Feb. 21	35.25	−10.70	Feb. 21	39.53	+0.06			
Feb. 26	38.21	−3.26	Feb. 28	39.70	+0.50			
Feb. 28	34.84	−11.79	Mar. 3	43.70	−10.64			
Mar. 3	38.17	−3.37	Mar. 7	46.76	+18.38			
Mar. 7	39.37	−0.33						
			Post-training					
Mar. 10	40.10	+1.52	Mar. 10	44.36	−12.30	Mar. 13	35.76	−9.47
Mar. 14	37.28	−5.62	Mar. 12	41.69	+5.54	Mar. 21	38.03	−3.73
Mar. 17	37.29	−5.60	Mar. 14	40.87	+3.47	Mar. 27	36.64	−7.24
Mar. 21	38.42	−2.74	Mar. 17	42.99	+8.83	Apr. 10	37.23	−5.75
Mar. 28	37.52	−5.01	Mar. 21	40.75	+3.16	Apr. 15	35.48	−10.17
Apr. 16	35.31	−10.60	Mar. 28	43.69	+10.61			
			Apr. 16	38.88	−1.57			
			May 16	37.73	−4.48			

some change in his basal metabolic rate, but the pattern of the change is variable. One subject experienced a marked rise, another a moderate rise, and the third a moderate drop in the rate.

Prior to training I. B. H. showed an average basal metabolic rate of −2.65 (see table 2). Throughout the entire period of training and for three weeks of the post training period his basal metabolic rate ranged between +0.06 and +18.38 per cent. His average for the training period was +7.76 per cent.

We were at first inclined to attribute the high metabolic rate of this subject to the after effects of the exercise of the previous day, Wisehart (1927) having found that an hour's work on the previous day raised the metabolic rate one to two per cent. Because of this I. B. H. was requested to omit exercise on the day prior to a metabolic determination. This had no effect on the result. Furthermore his post-training data (see table 2) show that the same high rate continued for some time after exercise was omitted. Later, approximately five weeks after the training period, his metabolic rate once more dropped slightly below the standard normal of DuBois.

Subject G. W. H. (see table 2), starting with an average basal metabolic rate of −7.87 per cent in the pre-training period, showed an average rise to −3.92 per cent during training and an average of −6.72 per cent in the post-training period.

Subject A. O. F. normally ranged around −6 per cent. During the first four weeks of training his basal metabolic rate was clearly lowered, averaging −11.46 per cent. Thereafter and for three weeks of the post-training period it was back to his normal.

It is apparent from these and past observations made in this laboratory that the basal metabolic rate of all men does not respond in the same manner to a period of physical training. Of seven non-athletes who have been under observation during periods of daily moderate physical exercise, three have shown an appreciable fall, two a rise, and two no change in the metabolic rate. Among ten athletes, eight have shown a drop in the basal metabolic rate during training, one an increase, and another no change in the rate.

The literature on this subject reveals that some workers in this field have reported that physical training causes a rise while others have reported that it causes a fall in basal metabolism. This literature has been briefly reviewed by Schneider, Clarke and Ring (1927) and need not be included here.

Can a physiological explanation be found for the diversity of results that has been observed in the study of the basal metabolic rate of athletes, non-athletes and animals in training? We believe that either a rise or a fall in metabolism may be explained on a physiological basis. Recognizing that metabolism is a cellular process, that each living cell uses its share of the total oxygen absorbed by the individual, it is evident that our real problem is to determine whether or not the cellular processes of the body

are accelerated, retarted, or unaltered by training. If the cells are stimulated to increased activity, they will use more oxygen; but if the life process becomes more efficient, they will use less oxygen. Training may so affect the cells that they will use, even in rest, more oxygen or less oxygen than formerly; or it may not alter their usage of oxygen. There is a widespread opinion that the body is physically and mentally more efficient if regular physical exercise is taken. While we can not at present prove that cellular metabolism becomes more efficient as a result of training, if we assume it does then an explanation is at hand for the fall in the basal metabolism seen in some athletes and non-athletes during a period of training.

If a period of physical training should at the same time result in an increase in the mass of active tissue in the body, then we must assume that the added protoplasm requires its share of oxygen and that this increases the total oxygen requirement of the body.

May not the above two reactions to training work against each other? If during a period of training the gain in cellular efficiency overbalances the gain in the mass of body protoplasm, then a fall in the basal metabolic rate will result. If on the other hand there occurs a considerable increase in the size of muscles, due to a formation of active protoplasm, then this may overshadow the gain in cellular efficiency and result in an increase in the basal metabolism. Lastly, if the increase in efficiency should just be counterbalanced by the new production of protoplasm, the basal metabolic rate of the individual would remain unchanged during a period of physical training.

That regular daily physical exercise may increase the amount of active protoplasm in the body can scarcely be questioned. But we may question whether the naturally athletic young man of college age ever so far neglects physical exercise as to permit a material amount of atrophy of his muscles. Very likely the athlete, as we find him in our colleges, does not develop more muscular protoplasm during a season of training. Hence the suggestion made by Steinhaus (1928) may account for a fall in the basal metabolic rate during training. From an investigation on dogs he suggests that the high degree of relaxation, which is acquired by trained animals, may explain why it is possible to have muscle hypertrophy without an increased metabolic rate. He believes that the athlete somehow learns to relax very well.

Very likely most of us are disposed to accept the statement of Bainbridge when he says, "The real value of exercise probably lies mainly in its effects upon the metabolism of the tissues themselves."

As regards the likelihood of an increase in the efficiency of cellular life a statement of Bock and co-workers (1928) is of interest when they comment upon the efficiency of the marathon runner, DeMar, who had maintained a state of training continuously for approximately twenty years.

During this time, with few exceptions he ran ten miles or more daily. They say "Although little of a definite nature is known about the mechanism of cell oxidation, we believe that the capacity for exercise is bound up with changes in the oxidative processes within the cell. A search for the secret of such efficiency as we have shown to exist in the case of DeMar does not yield an answer in terms of increased efficiency of breathing or circulation alone. It appears to lie rather in the muscle cells themselves."

SUMMARY

1. The basal metabolic rate was lowered in seven, unchanged in one, and elevated in another athlete during training.

2. A period of a moderate amount of physical training raised the metabolic rate in two and lowered it in a third non-athlete.

3. A physiologic explanation is suggested for the varied influence of physical training.

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BILE FLOW AND BILE PIGMENT OUTPUT AFTER DENERVATION OF THE LIVER

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The liver, as the other viscera, has a double innervation from the sympathetic system and from the parasympathetic system. Most of the nerves that reach the liver are sympathetic in origin, coming from the celiac plexus. Small branches from each vagus nerve go to the liver and some investigators also have noted small nerve bundles going from the right phrenic nerve. All the nerves to the liver, except a small branch from the left vagus, accompany the hepatic artery to the organ. The intrahepatic distribution of the nerves is not definitely known. On the whole, the nerve supply to the liver is generous, and would appear to be of considerable physiologic significance. It has not been proved that the nerves to the liver serve any other purpose than that of vasomotor function. However, there are several well-known phenomena which could well be considered as indicating an influence of the nervous system not mediated through the vascular system, on hepatic activity. Among these phenomena can be mentioned the following: 1, operative procedure on the biliary tract is often followed by striking decrease in flow of bile; 2, excitement of an animal with a biliary fistula often will be followed by decrease in flow of bile and 3, ingestion of food is usually followed by increase in flow of bile. As a preliminary investigation in this laboratory, to determine whether there are nerves to the liver which directly affect either the secretory function or other functions of the organ, a study of secretion of bile following denervation of the liver was made.

Several investigations have been performed in a study of the effect of the hepatic nerves on secretion of bile. The majority of these investigations have dealt with the effect of stimulation of the sympathetic or vagus nerves, of section of the spinal cord or of injection of various drugs. The change noted in the flow of bile could be ascribed to effects of the procedure, or of drugs employed, on flow of blood through the liver or on the biliary tract. Recently, Gaissinsky noted increase in the cholesterol content of the bile after section of the sympathetic nerves to the liver, but did not note a change when the vagal supply was severed. Experiments on the

flow of blood from the liver, by Burton-Opitz, Macleod and Pearce, and plethysmographic observations on the liver, by Mattson, have amply demonstrated the vasomotor control of flow of hepatic blood.

METHODS. All of the experiments were performed on dogs. The animals were given a standard mixed diet consisting of ground meat, dog-biscuit and milk, which was fed in the same amount and at the same time every day. They were trained to lie quietly with slight restraint. Under ether anesthesia, and employing sterile technic, the gall bladder was removed and a cannula was inserted into the common bile duct making a modified Rous-McMaster's type of biliary fistula. Observations were then carried out on the rate of flow of bile and the output of bile pigment was estimated. Specimens were collected and measured every two hours. After a sufficient number of control observations had been made, the liver was denervated. To accomplish this all structures in the lesser gastro-hepatic omentum, except the common bile duct, hepatic artery, and portal vein, were severed. Owing to the presence of the cannula it was not always possible to be sure that the branch from the vagus was sectioned, and it was therefore sometimes necessary later to section both vagus nerves in the thorax in order to be certain that the vagal supply to the liver was severed. The concentration of bilirubin was estimated by the van den Bergh method.

RESULTS. Observations were carried out on seven dogs. The animals remained in good condition during the time they were under observation. In the first few days the bile was concentrated and the amount of bile was small. Three to four days after the fistula had been established, an average of about 50 cc. of bile was secreted in six hours. Ten days after operation the output of bile pigment became stabilized, on the diet given, at 60 to 80 mgm. for twenty-four hours. The effect noted following feeding was the same as that reported elsewhere.

Very little change was noted in the biliary secretion and in the output of bile pigment following denervation of the liver. From one animal there was a definite decrease in output of bile pigment after the second operation, but no effect was observed on the other animals. The result in the one animal can hardly be ascribed to the denervation of the liver.

SUMMARY

Observations were made on the flow of bile and on the output of bile pigment before and after denervation of the liver. Denervation of the liver did not produce a constant effect either on the amount of bile secreted or on the amount of pigment eliminated.

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STUDIES ON CARBOHYDRATE METABOLISM FOLLOWING DENERVATION OF THE LIVER[1]

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The most important known function of the liver is the regulation of the concentration of sugar in the blood. The mechanism whereby the concentration of the sugar in the blood is kept fairly constant is not known.

Interest in the relationship of the nervous system to carbohydrate metabolism first began when Claude Bernard (1849) punctured the floor of the fourth ventricle in animals and produced hyperglycemia and glycosuria. From further studies he concluded that piqure (puncture) hyperglycemia was due to impulses passing from the medulla, down the spinal cord, out through the splanchnic nerves to the celiac plexus, and finally to the liver. The Cavazzani brothers (1893) first stimulated the celiac plexus in dogs, and demonstrated hyperglycemia and reduction in hepatic glycogen. Macleod (1907) produced similar hyperglycemia by stimulating the left great splanchnic nerve. Blum (1901) discovered that epinephrine administered subcutaneously or intravenously, caused hyperglycemia and glycosuria. Since that time much experimental work has been done in an attempt to determine the relative importance of epinephrine and of the nervous system in regulating the level of blood sugar. Emotional hyperglycemia, reflex hyperglycemia as produced by stimulation of the central end of a sensory nerve, and asphyxial hyperglycemia are all attributed to increase in liberation of epinephrine into the blood stream and its action on the liver in causing increase in blood sugar. At the present time, the only known function of the hepatic nerves is the vasomotor function. It has been suggested that hyperglycemia resulting from administration of epinephrine, or stimulation of nerves is due to vasoconstriction.

The purpose of this investigation was to determine whether denervation of the liver produced any changes in the response to the readily available substances which uniformly cause either hyperglycemia or hypoglycemia.

[1] Abstract of thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment for the degree of Master of Science in Surgery.

METHOD. All experiments were carried out on dogs. The care of the animals was standardized, including the diet and the period of fasting before observations were made. The response of the animals to intravenous administration of glucose, epinephrine, extract of the posterior lobe of the pituitary body, and insulin, in regard to changes in blood sugar, were determined. After a sufficient number of curves of blood sugar were obtained following administration of each substance in the normal animals, the liver was denervated. All operations were performed under ether anesthesia, employing sterile technic. The liver was denervated as described by Lundberg. In some animals the vagus nerves were also sectioned in the thoracic cavity. The method of Folin and Wu for determination of sugar was used.

To permit recovery of the animal from the operation, observations were not made until the seventh to the tenth day following denervation of the liver. The animals were then given a diet similar to the one used before operation. Experiments were performed in a manner similar to that employed before denervation. Observations were made at intervals over a period of four months after denervation.

RESULTS. *Fasting blood sugar level.* The average normal fasting blood sugar level of the dogs used in this study ranged between 93 mgm. and 103 mgm. in each 100 cc. of blood. The animals were fasted sixteen to twenty-four hours before each experiment. There was no essential difference in the fasting blood sugar level before and after denervation of the liver.

Epinephrine hyperglycemia. Epinephrine was administered intravenously in doses of 0.05 mgm. for each kilogram of body weight. It was diluted with 10 cc. of physiologic sodium chloride solution and the mixture was injected slowly into the external jugular vein. A sample of blood was obtained before injection of epinephrine and at standard intervals afterward for two or three hours. The concentration of blood sugar began to rise within three minutes after injection of epinephrine and reached its maximal value usually within the first six minutes. Following the maximal rise the value for blood sugar rapidly returned to normal or slightly above normal. Epinephrine hyperglycemia is essentially the same before and after denervation of the liver. The average maximal rise was 52 per cent before denervation and 60 per cent after denervation (fig. 1).

Glucose tolerance test. Glucose was given intravenously to determine its rate of disappearance from the blood stream. One cubic centimeter of glucose solution, 38 per cent for each kilogram of body weight was administered. Samples of blood were taken before injection and at standard intervals for two hours afterward. Denervation of the liver did not affect the curve of glucose tolerance (fig. 2).

Hyperglycemia due to extracts of the posterior lobe of a pituitary body. The

hyperglycemia following intravenous injection of extracts of the posterior lobe of the pituitary body, "Pituitrin" and "Vasopressin" was determined before and after denervation of the liver. Pituitary extract was given in

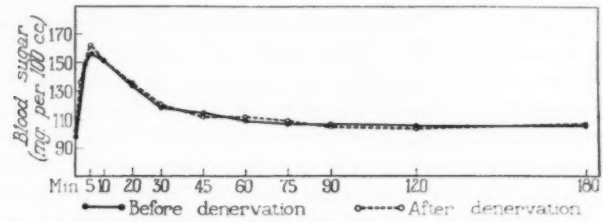

Fig. 1. Hyperglycemia caused by epinephrine before denervation of the liver (average of thirteen curves recording observations on five dogs). Broken line: hyperglycemia caused by epinephrine after denervation of the liver (average of twenty-three curves recording observations on the same five dogs). Studies were made over a period of four months after denervation.

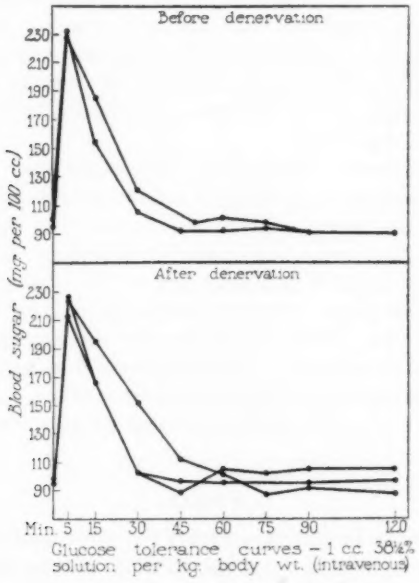

Fig. 2. Curves recording glucose tolerance before and after denervation. Studies were made over a period of four months after denervation.

doses of 1 cc. diluted in 10 cc. of physiologic sodium chloride solution. It was necessary to give pituitrin and vasopressin in smaller doses. The hyperglycemic response of pituitary extracts is somewhat similar to that

of epinephrine. The maximal rise in concentration of blood sugar is reached in about ten minutes and it drops more slowly than it does following injection of epinephrine. This type of hyperglycemia was not affected by denervation of the liver.

Insulin hypoglycemia. Insulin was administered intravenously in doses of 0.5 unit for each kilogram of body weight, and diluted in 10 cc. of physiologic sodium chloride solution. A specimen of blood was removed before injection, and at standard intervals afterward, for two hours. The average maximal drop in the level of blood sugar was 38 per cent, occurring within the first fifteen minutes after injection. It remained at this level, or slightly lower, for forty-five to sixty minutes, when it gradually began to rise. The hypoglycemia was essentially the same before and after denervation of the liver.

COMMENT. The relationship of the nervous system to metabolism of carbohydrate is not definitely known. Most of the previous work on the subject has dealt with changes in metabolism of carbohydrate produced by stimulation of those parts of the nervous system which contain nerve fibers which supply the liver; for example, changes caused by piqure, stimulation of the splanchnic nerves, celiac plexus, and the hepatic plexus. Previous work on the effect of denervation of the liver has been in the form of acute experiments, before the nerves peripheral to the line of section have had time to degenerate.

In the present work, observations were made over a period of four months after denervation of the liver, allowing ample time for complete degeneration of the nerves. There was no essential difference observed in the level of blood sugar before and after denervation. Studies were made on the value for fasting blood sugar on the hyperglycemia produced by epinephrine, pituitary extracts and glucose, and on the hypoglycemia produced by insulin. Denervation did not change the effect of these substances on the blood sugar. Hyperglycemia from epinephrine does not depend on an intact nerve supply to the liver. It has been shown that its vasoconstricting action likewise does not depend on an intact nerve supply to an organ.

SUMMARY

Denervation of the liver affects neither the value for fasting blood sugar, the hyperglycemia produced by epinephrine, glucose and pituitary extracts, nor the hypoglycemia produced by insulin. Accordingly, this study would seem to indicate that the regulation of the value for blood sugar does not depend on an intact nerve supply to the liver.

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THE FORMED ELEMENTS OF THE BLOOD, COAGULATION TIME, PEPTONE LEUKOPENIA AFTER SECTION OF THE NERVES TO THE LIVER

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As the nerves to the liver are apparently vasomotor in function, it has been natural to consider that they would be factors in the control of certain constituents and processes in relation to the blood and to circulation. The leukopenia which occurs after administration of peptone is due mainly to reduction in number of polymorphonuclear leukocytes. It has been suggested that this leukopenia is due to accumulation of the leukocytes in the vessels which are controlled by the splanchnic nerves, especially the vessels of the liver (1), (4), (5). Probably on the basis of evidence that the liver is significant in the formation of fibrinogen it has been suggested that the hepatic nerves are significant in the process of coagulation. Griffith and Friedman sectioned the nerves in the hepatic pedicle of cats and noted a tendency to bleed for a considerable time after operation. Finally, evidence has been presented to show that the blood sinusoids of the liver act somewhat in the capacity of a storehouse for erythrocytes.

In view of the contradictory observations in regard to the effect of the hepatic nerves on certain constituents and processes associated with the blood, it seemed desirable to investigate further. The attempt was made to determine whether the hepatic nerves have any effect on the formed elements of the blood, on coagulation time or on peptone leukopenia.

Methods. The experiments were carried out on cats and on dogs. All operative procedures were performed under ether anesthesia, employing sterile technic. Control data in regard to the number of erythrocytes and leukocytes, including differential count of the latter, coagulation time of the blood, and estimations of hemoglobin were obtained. After sufficient observations had been made the nerves to the liver were sectioned after the manner described by Lundberg. Observations were then repeated at frequent intervals until the termination of the experiment which varied in time from one week to two and a half years. The method of following the peptone leukopenia was as follows: The animal was etherized and a lobe of liver exposed. Leukocytic counts were made of blood from a small

stab wound of the liver and of blood from a peripheral vein. Then 10 cc. of 20 per cent solution of peptone were injected intravenously. When the effect of peptone was manifested, leukocytic counts from the same or similar sites were again made. Occasionally counts were also made from the blood of a mesenteric vein before and after injection of peptone. These observations with peptone were carried out on normal cats as well as on cats in which the nerves to the liver had been sectioned previously.

RESULTS. The results of all experiments were negative. Section of the nerves to the liver did not change the number of erythrocytes, the number of leukocytes, or the coagulation time of the blood. A decrease in the number of leukocytes in blood from the liver and peripheral vein occurred in the denervated animals as well as in the control animals after the injection of peptone.

SUMMARY

The number of erythrocytes, the number of leukocytes, and the coagulation time of the blood were determined before and after section of the nerves to the liver. Denervation of the liver did not produce any effect on the factors studied. Furthermore, denervation of the liver did not affect the action of peptone in decreasing the number of leukocytes in the blood from the liver.

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THE EFFECT OF DENERVATION OF THE LIVER ON SECRETION OF BILE

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Although the liver secretes bile continuously, the rate of secretion is subject to considerable variation. It is commonly known that the secretion of bile is greatly diminished by fasting, and greatly increased by the ingestion of food. The increase following the taking of food may be due to several factors, among which may be considered the hepatic nerves. The nerve supply to the liver is generous. The purpose of this investigation was to determine whether the increased flow of bile associated with increased activity of the digestive tract is due to a nervous reflex.

EXPERIMENTAL METHODS. All operations were performed on dogs, under ether anesthesia, using strict aseptic technic. In some of the experiments a total (common duct) bile fistula was made, as pointed out by Lundberg. In others a cannula was introduced and tied into one of the hepatic ducts; thus only the bile from two lobes of the liver was drained to the outside; the bile from the remaining lobes was allowed to enter the intestine as usual.

Operations of denervation were performed as a second stage on the same animals; they were done as described by Lundberg. However, it was often technically impossible to section all the nerves to the liver without disturbing the cannula. All nerves in the hepatic pedicle were sectioned in all cases but the branch coursing directly from the vagus to the liver was not always severed. Therefore, other experiments were done as controls. In these, the liver was denervated and a cannula was inserted at the same operation.

Each animal was observed with regard to the effect of feeding on the secretion of bile. In the first series of experiments (two-stage operation) observations were made both before and after denervation of the liver; in the second series (one-stage operation) they were made necessarily only after denervation of the liver. The animals were trained to lie on well padded, specially constructed tables, and bile was collected for fifteen-minute periods and measured. Four such readings were taken on the fasting animal, to determine a true fasting level of secretion for that par-

ticular day. Then a test meal was given, and fifteen-minute collections of bile continued for at least three hours.

Standardized test meals were used as follows: 1, protein meal, 350 cc. condensed milk; 2, protein meal, 400 grams raw lean meat; 3, fat meal, 240 cc. cream and two egg yolks; 4, carbohydrate meal, 150 cc. syrup and 100 grams bread, and 5, mixed meal, 200 grams raw lean meat, 100 grams dog biscuits, and 100 cc. skimmed milk.

RESULTS. Observations made on the first series of animals, those with total or partial bile fistulas on which determinations were made before and after denervation of the liver, were as follows:

Before denervation of the liver, after a sixteen-hour fast, the bile was scanty and concentrated. However, collections made every fifteen minutes for four hours demonstrated that the rate of secretion was fairly

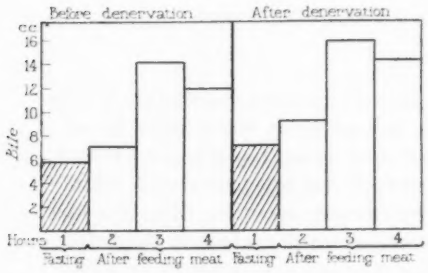

Fig. 1

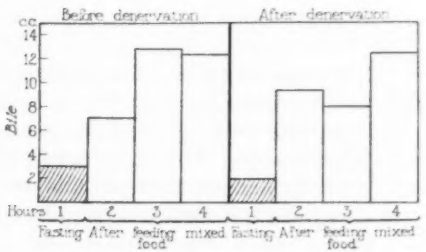

Fig. 2

Fig. 1. The effect of feeding meat on bile secretion before and after denervation of the liver.

Fig. 2. The effect of feeding meat, dog biscuits, and milk on bile secretion before and after denervation of the liver.

constant. But if, after collecting fasting specimens for one hour, the animal was then given one of the foregoing test meals, the bile collected during the next three hours showed marked changes. Usually there was a latent period for fifteen or thirty minutes after feeding, in which the bile continued to be secreted at the fasting level, or even diminished. Occasionally there was no latent period; the rate of secretion rose abruptly immediately after feeding. The quantity of bile secreted in the first hour after feeding was usually somewhat greater than the amount secreted during the hour of fasting. The quantity secreted in the second hour after feeding was usually the largest, and the quantity during the third hour was slightly less. A meal of meat, milk, and dog biscuits usually caused secretion of bile to be doubled, tripled or quadrupled. A meal of raw lean meat usually caused the secretion to be doubled or tripled, and

in one case quadrupled. A meal of condensed milk usually caused the secretion to be doubled, and occasionally tripled. A meal of egg yolks and cream usually caused an increase in the secretion, the rate being one and a half to two times the fasting level; occasionally, however, there was neither increase nor decrease. A meal of bread and syrup usually caused the secretion to rise to one and a half to two times the fasting level; often, however, there was no change.

It was found that after denervation of the liver, as before denervation, the fasting rate of secretion was fairly constant. Each of the various test meals produced essentially the same increase in biliary secretion as had occurred before denervation, or even slightly greater in a few instances.

The results of experiments in which the denervation was done when the fistula was made were identical with those obtained before and after denervation. The section of the nerves to the liver did not prevent the increased secretion of bile which follows the ingestion of food.

SUMMARY

A study was made to determine whether the increase in secretion of bile which follows the ingestion of food is dependent on the hepatic nerves. Five dogs were operated on, the gallbladder being removed and biliary fistulas made. After a sixteen-hour fast each dog was placed on a collecting table and specimens of bile were collected as controls. The dog was then fed a test meal and bile was collected at regular intervals thereafter. It was found that a mixed meal caused the greatest increase of biliary secretion, a protein meal caused a marked increase, a fat meal usually caused a moderate increase, occasionally there was neither increase nor decrease, and a carbohydrate meal gave variable results, either an increase or a slight decrease, or the output remained the same.

After these control observations had been made on the animals, the hepatic nerves were sectioned. Fasting, feeding, and collections were carried out as before, and it was noted that each of the foregoing test meals produced about the same increase in biliary secretion as before denervation. These observations show that the increase in biliary secretion after the ingestion of food is not dependent on the nerves to the liver.

HEAT REGULATION AND WATER EXCHANGE

XIII. Insensible Weight Loss and Liver Hydration in the Onset of Cocaine Fever

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Recent work from this laboratory has shown that during the onset of cocaine, beta-tetra, anaphylactic (Barbour and Marshall, 1931) and neurogenic fevers, (Barbour and Aydelotte, 1931) the liver stores a considerable amount of water. This withdrawal of water with its accompanying anhydremia occurs just when there is extra demand for water in the processes of heat elimination. It becomes important, therefore, to learn to what extent, if any, actual interference with the surface elimination of water occurs.

It is true that we may look upon either type of perspiration as largely independent of the existing water supply. For example, Geréb and Laszlo (1931) have shown that insensible perspiration can be influenced but very little by the Volhardt method of drinking excess amounts of water, either in the blood dilution or concentration phase. Nevertheless it cannot be questioned that, as in the case of water loss through the kidney, the hydration of the blood must attain a certain minimum if water is to be yielded for surface evaporation.

The possibility that the onset of fever exhibits water shifts other than between liver, blood and skin, has received much of our attention. Cocaine fever in rabbits was especially studied and is regarded as fairly typical of the febrile condition in general. Here the rabbit was found to exhibit no change in the water content of brain, muscle or kidney. Furthermore, we have sought in vain for fluid accumulation in the pericardial, pleural or peritoneal cavities. Evidence of water shifting not mentioned above was limited to a possible dehydration of the skin. As regards the possibility of extra water loss from the body, no evidence was found of increased salivation, diarrhea or diuresis. There is no evidence in the literature to indicate that cocaine acts as a diuretic under any conditions; indeed

[1] The experimental data in papers XII and XIII are taken from the thesis of Hubert T. Marshall, presented in candidacy for the degree of Master of Science, at the University of Louisville, 1930.

the work of Maestro (1904) indicates that the urinary output of rabbits is decreased by cocaine.

Our primary objective in the present work has been, therefore, to outline more clearly the general relationships obtaining between water storage by the liver, anhydremia and insensible weight loss.

The insensible perspiration cannot be considered apart from the total metabolism. Benedict and Root (1926) have demonstrated for normal man such a close relationship between insensible perspiration and metabolic rate that the former, with due reservations, may be used as an index of the latter. In a variety of pathologic conditions, however, the relation of insensible loss to total metabolism is not at all constant, as has been shown, for example, by Laszlo and Schürmeyer (1931) in various non-febrile connections.

In the rabbit the insensible perspiration has been extensively studied by André Mayer and Nichita. In this animal water is vaporized not only from the lungs, but also (although sweat glands have never been found) from the skin (Keeton, 1923). Mayer and Nichita (1929), extending Rubner's work, have found under constant environmental conditions, that the water loss is influenced systematically by the respiratory exchange. They have shown that the H_2O/O_2 ratio (water lost per oxygen consumed) is constant not only for the same individual at different times, but also for rabbits in general. At a room temperature of eighteen degrees Centigrade, this ratio amounts essentially to unity. When the metabolism is stimulated as by strychnine, methylene blue, pilocarpine and other agents, the insensible loss increases faster than the metabolism. Consequently the H_2O/O_2 ratio becomes increased. Since this implies a greater increase in the heat loss by vaporization than in the total calories produced, radiation, etc., must be decreased, for a rise in body temperature occurs. In such instances of fever the authors appear to assume that the water loss is entirely dependent upon the metabolic rate; but it must be noted that the minimum length of the febrile periods studied was two hours, thus obscuring the events of the first half-hour, the importance of which we shall now indicate.

In cocaine fever it has become evident to us that the maximum hydration of the liver occurs within the first half-hour. A time curve of this process corresponds roughly to a similar curve of water lost from the blood. In our previous paper it was stated that about 5 cc. water were gained by a sixty-gram liver during the early part of cocaine fever; this merely average figure applies to the condition found in the liver of rabbits killed about one hour after the injection. That there is a considerably greater hydration of the liver during the first half-hour, is shown by the following increases in liver water which we have now calculated for various intervals after cocaine injection: 15 per cent, twenty minutes; 11.5 per cent, thirty minutes (average of two rabbits); 9.5 per cent, forty minutes (average

of two rabbits); 8 per cent, sixty minutes (average of five rabbits); 7 per cent, ninety minutes; 3 per cent, 120 minutes.

During the first half-hour, therefore, the sixty gram liver takes up from 7 to 9 cc. of water, which apparently exceeds the amount lost by the blood. Hence during the onset of fever water seems to be diverted to the liver from some further source, presumably the skin and subcutaneous tissues. By the end of two hours however the extra water has nearly all gone away again, apparently leaving at a steady rate of 3 to 4 cc. per hour.

The questions arise: What has heat loss by vaporization to do with the above water shift? Is the liver action during the crucial first half-hour, sufficient to reduce the heat elimination? Or if not, can it at any rate prevent the insensible loss from increasing at that time?

PROCEDURE. Our studies of the insensible loss during cocaine fever in rabbits were made under conditions similar to those obtaining during the liver investigations. In addition to the weight loss, which was studied in five cocaine poisoned rabbits, the respiratory exchange was followed in four of these animals, affording not only a minor correction of the insensible loss for the gaseous exchange, but also making it possible to relate the water loss to the heat production.

The experiments were conducted in a small, insulated room (capacity six cubic meters) where temperature and humidity could be kept nearly constant. These atmospheric conditions were recorded at half-hour intervals and in most experiments the wet-kata cooling power was simultaneously determined. When this stood between sixteen and nineteen millicalories per square centimeter per second, the conditions were found most satisfactory; cooling powers of fifteen millicalories and below tend to produce hyperthermia in rabbits. Circulation of air was maintained by a small fan, never blown directly upon the animal. Rabbits of the same stock and age as those employed in our liver solids work were used; insensible loss (body weight and gaseous exchange), respiratory volume and rectal temperature were determined.

The rabbits were weighed in a light wire basket, on a balance sensitive to 0.1 gram. In most experiments weights were taken every half-hour,—in a few cases hourly. The bladder and rectum were emptied by careful massage at the beginning of each experiment. A pan suspended under the basket facilitated the detection of evacuation; if this occurred a new period was started. In none of the experiments described in this paper were urine or feces evacuated after cocaine administration. Rectal temperature readings were taken with great care to avoid altering the body weight.

In determining the respiratory exchange the expired air was collected in a very light rubber bag, the inflation of which offered minimal resistance to expiration. A mask, made from the light metal cap of a thermos bottle, was fitted tightly over the rabbit's face at least an hour before beginning the collection of samples for analysis. This mask was connected through a T-tube to the rubber bag. The inspiratory and expiratory valves were made of sausage casing as described by Pearce (1930). After the excitement of applying the mask had subsided the animal always became quiet and appeared to breathe normally, without effort.

After the animal had thus remained quiet for at least an hour the experiment was begun. The body weight was taken with the mask in place, the rectal temperature recorded and the T-tube connected with the mask. The animal was allowed to

breathe through the valves for a few minutes, after which expired air was collected in a basketball bladder until it was about half-full. It was then disconnected and the large soft rubber bag connected to the T-tube. The expired air was collected in this bag for exactly fifteen minutes, after which the partly filled basketball bladder was again substituted. Filling was completed and samples taken for immediate analysis. Thus a few minutes remained in each period for weighing and taking the rectal temperature.

To obtain the volume of expired air the contents of the light rubber bag were run through a gas meter. The volume for the half-hour period was assumed to be just twice the amount measured, while the samples from the basketball bladder were considered representative of a mixed sample of expired air for the period.

Following two or more normal periods and just after a final normal rectal temperature reading the cocaine was injected and the animal followed for several more periods. The exact weight of the cocaine solution injected was taken into account at the next weighing.

Samples of expired air were analyzed immediately after collection to preclude possible changes in composition due to slow diffusion through the walls of the basketball bladder. Each sample was analyzed in duplicate according to Haldane and Henderson (1918).

Both insensible loss and metabolism were calculated in terms of surface area, calculated by the Meeh-Rubner (1879) formula: $0.125 \sqrt[3]{W^2}$, where W represents the body weight.

The dose of cocaine used in this work varied from twenty to thirty milligrams per kilo, which gave prompt and typical reactions (the weather being warmer than when forty milligrams were required, as in paper XII). Feeble and very brief convulsions were sometimes observed in addition to the febrile reaction.

Results. Typical effects of cocaine upon the metabolism and insensible loss of the rabbit may be illustrated in figure 1. A 2.2 kilo rabbit (expt. 3) having a surface area of 0.21 sq. m., was given cocaine hydrochlorid 27.3 mgm. per kilo, at the end of the second hour of the experiment. It will be seen that a slight increase in the room temperature (from 30° to 30.8°C.) throughout most of the experiment, was compensated by a decrease in the relative humidity (40 to 38 per cent) so that the cooling power of the environment varied only between 15.5 and 16.8 millicalories per square centimeter per second. The first half-hour after cocaine the temperature rose 0.9°C. (from 39.6° to 40.5°C.), the maximum rise observed (after two hours) being 1.9°C. The metabolic rate rose 14 per cent during the first half-hour, viz., from 31.78 calories to 36.36 calories per square meter per hour. A maximum of 41.85 calories was reached in the third half-hour. The expired air volume followed the metabolism closely.

Until the fourth half-hour when the effects of hyper-ventilation became evident, the respiratory quotient remained at the fasting level of 0.71 to 0.74. At this level the correction of the insensible loss for the gaseous metabolism is quite negligible (because R.Q. = 0.73 represents approximately one gram of CO_2 for each gram of O_2). The body weight changes are

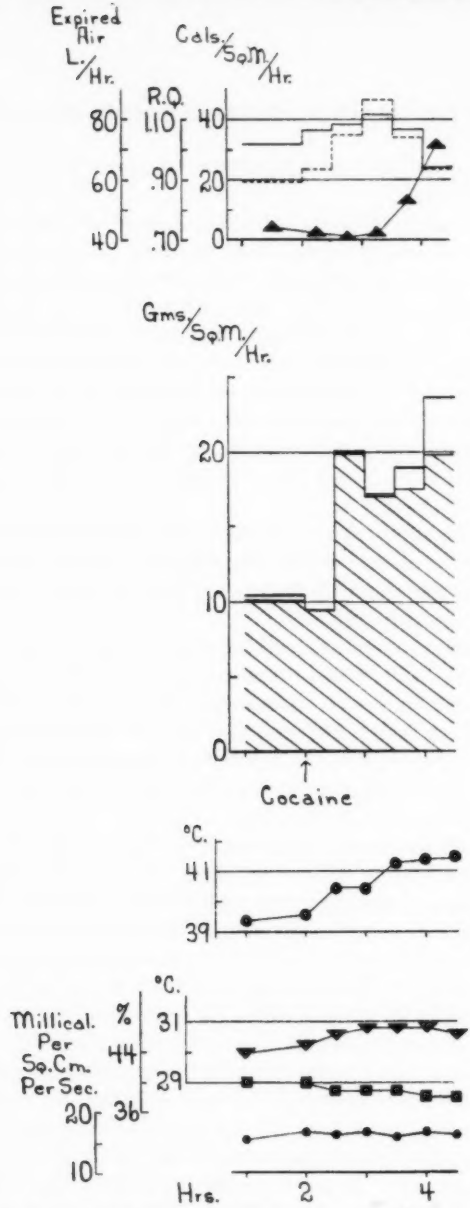

Fig. 1. Effects of cocaine hydrochlorid 27.3 mgm. per kilo, on metabolism and insensible loss of rabbit. Abscissae: hours p.m.; ordinates: metabolic features or room conditions.

Upper part: Continuous lines: total metabolism; broken lines: expired air; triangles: respiratory quotient.

Middle part: Upper lines: insensible weight loss; shaded area: water loss (when space intervenes this represents the correction for gaseous metabolism). Cocaine injected at arrow. Large dots: rectal temperature.

Lower part: Triangles: room temperature; squares: relative humidity per cent; small dots: wet-kata cooling power.

illustrated in the mid-portion of the figure, the corrected water loss being shown by the shading.

The essential feature of the experiment is seen in the first half-hour after cocaine, where, in spite of the metabolic increase, there was no rise in the water loss. Even a slight decrease took place (from 10.16 to 9.45 gm./sq. m./hr.). It will be recalled that during this period the liver exhibits its maximum hydration and the blood its maximum dehydration. After the first half-hour and for the next two hours the total water loss was almost double its basal volume (varying between 17.12 and 20.17 gm./sq. m./hr.); this is the period of steady decline in liver water. It is significant that during these two hours' vaporization water was lost in excess of the basal at a steady rate of about 3.5 cc. per hour; this agrees with the calculated rate of water loss from the liver.

The ratio of the vaporization calories to the total calories, as calculated from the data of the above described experiment, fell from 0.19 to 0.15 during the first half-hour, suddenly rising to 0.31 for the second half-hour, after which a high level was maintained.

The details of experiment 3 should be compared with those of four others, shown in the table. Time and room conditions are given in the first four columns, after which are seen successively the changes in rectal temperature, respiratory quotient, total insensible loss, water loss, resulting calories lost by vaporization and total calories of the metabolism. The final two columns show respectively the percentage increase in the total calories and the vaporization:metabolism ratio.

Experiments 3 to 5, it will be noted, although the environmental cooling power was definitely lower, did not yield results significantly at variance with those of the other two experiments. In experiment 4, however, the low cooling power was evidently sufficient to induce some hyperthermia (40.8°C.) in the rabbit before the cocaine was injected.

Insensible loss and its relation to the total metabolism. The total insensible loss, as the table shows, was definitely lowered after cocaine in experiments 1 and 3. In the latter case, it was increased after the first half-hour, as described above. In experiment 1, however, although no correction is made for the gaseous metabolism, it seems certain that for three hours after cocaine there was a much lower rate of water loss than before the injection. This was the smallest dose of cocaine injected and the least effective so far as fever was concerned. In one experiment (5) there was a slight increase in the water loss during the first half-hour after cocaine, while in experiments 2 and 4, the total insensible loss and the water loss were both decidedly increased even during this period.

The variability of these results might, at first sight, lead to doubts as to the existence of an influence working against surface vaporization during the onset of fever. But we must consider the opposing rôle of the metabo-

TABLE 1

TIME	ROOM TEMPERATURE	RELATIVE HUMIDITY	COOLING POWER	RECTAL TEMPERATURE	R.Q.	INSENSIBLE LOSS		LOSS BY VAPORIZATION	TOTAL METABOLISM	METABOLIC INCREASE	RATIO VAPORIZATION CALORIES: TOTAL CALORIES
						Total	H_2O				
Experiment 1. Rabbit 2.2 kilos. Surface area 0.21 sq.m.											
	°C.	per cent	millical/sq.cm./sec.	°C.		gms./sq.m./hr.	gms./sq.m./hr.	calories/sq.m./hr.	calories/sq.m./hr.	per cent	
1:00	24.5	41		38.55							
2:00	25.0	42		38.80		11.37					
3:00	25.0	42		38.90		12.56					
3:01	Cocaine-HCl 20 mgm. per kilo										
4:00	25.0	42		39.65		9.00					
5:00	26.0	40		39.80		9.95					
6:00	25.2	43		40.00		9.00					
Experiment 2. Rabbit 2.8 kilos. Surface area 0.25 sq.m.											
1:50	24.0	39		39.65							
2:50	24.0	39		39.40							
3:50	24.5	39		39.45	0.81	13.05	11.89	6.95	30.74		0.23
3:55	Cocaine-HCl 20 mgm. per kilo										
4:50	25.0	40		40.80	1.04	22.40	16.99	9.92	49.27	60	0.20
5:50	25.0	41		39.65	0.82	28.80	27.42	16.00	36.82	20	0.44
Experiment 4. Rabbit 2.2 kilos. Surface area 0.21 sq.m.											
12:45	30.2	39	14.7	40.45							
1:45	30.5	39	16.6	40.80	0.73	11.37	11.30	6.60	26.84		0.25
1:46	Cocaine-HCl 27.3 mgm. per kilo										
2:15	30.6	39	15.6	41.35	0.71	19.91	20.21	11.80	47.12	76	0.25
2:45	30.8	39	15.0	42.20	0.71	25.60	25.75	15.00	43.40	62	0.35
3:15	30.8	39	15.0	42.55	0.72	43.61	43.62	25.20	36.60	36	0.69
3:45	31.0	38	15.1	42.85	0.77	36.02	35.36	20.60	36.18	35	0.52
4:15	31.0	37	14.6	43.30	0.74	23.70	23.49	13.70	29.40	10	0.47
4:45	30.9	37	15.6	43.40	0.88	19.91	16.69	9.80	50.26	87	0.20
5:15	30.8	37	15.1	43.90	0.92	21.80	19.29	11.30	32.60		0.35
Experiment 5. Rabbit 2.0 kilos. Surface area 0.20 sq.m.											
10:10	29.8	50	18.2	40.10							
10:40	29.8	50	15.1	40.00	0.70	11.11	11.32	6.60	25.63		0.26
10:43	Cocaine-HCl 30 mgm. per kilo										
11:10	29.5	50	17.8	40.90	0.81	14.14	12.76	7.50	37.90	48	0.20
11:40	29.8	50	15.4	42.05	1.58	71.71	59.55	34.80	36.26	41	0.96
12:10	30.0	54	16.5	43.10	0.90	15.15	12.56	7.30	37.27	45	0.20
12:40	29.8	59	18.7	43.90	0.96	31.31	28.04	16.40	34.85	36	0.47

lism. Like Benedict and Root in man, Mayer and Nichita have, in rabbits, extensively demonstrated the parallelism between metabolism and insensible loss, and have included further the $H_2O:O_2$, which we prefer to express as vaporization calories/total calories.

Now after giving fever agents to rabbits, Mayer and Nichita paid no special attention to the initial period of abrupt onset; their first post-injection period always consisted of two or more hours. When this period is taken as a whole our work quite confirms for cocaine fever, the findings of Mayer and Nichita with strychnine, methylene blue, pilocarpine, etc. A glance at our figure and table will show the marked febrile increase in metabolism and the still more rapid increase in the water elimination.

Applying the vaporization calories/total calories ratio to the first half-hour of our experiments, the influence working in all cases against liberation of water now becomes clear. The two experiments in which the absolute water loss was increased, were those presenting the most marked increases in total metabolism during the first half-hour,—sixty and seventy per cent respectively. Taking into account these changes in the total metabolism, and those in the other experiments, the behavior of the water loss becomes consistent. *No increase in the ratio of vaporization calories to total calories ever took place during the first half-hour.*

In fact, with the exception of experiment 4, this ratio was always decidedly decreased. Although the metabolism was not done in experiment 1, there can be no reasonable doubt of a fall in the vaporization : metabolism ratio here also. In experiment 2, the water calories fell from twenty-three per cent to twenty per cent, in experiment 3, from nineteen per cent to fifteen per cent, in experiment 4, remained unchanged at twenty-five per cent, and in experiment 5, fell from twenty-six per cent to twenty per cent.

Turning to the second period after cocaine, we find in each of the four cases where the metabolism was determined a notable increase in the water loss both absolutely and in relation to the total metabolism. (In experiment 2 where the smallest dose of cocaine was used the second period coincided with the second hour and the abatement of fever.) The vaporization percentage ranged from thirty-one to forty-four in three experiments. While the ninety-six per cent calculated for experiment 5 may be an excessive figure, the unusual water loss of 59.55 grams was noted at the time and the weight carefully re-checked. The "apparent" respiratory quotient of 1.58 was furthermore an independent, simultaneous finding, so that little doubt remains of a tremendous but brief exaggeration of metabolic activity in this animal, somewhat compensated by a reaction in the period just following. The vaporization : metabolism ratio then returned to a high level like that maintained by the other animals during the continuation of fever.

The seat of increased water loss during fever in rabbits. In view of Keeton's demonstration of the significance of the skin in the insensible weight loss of rabbits, it is important to find out whether the skin is capable of increasing this function during fever for the excess water loss occurring after our first half-hour period, simultaneously with an increase in the expired air volume, might conceivably be due essentially to an increase in the amount of water vaporized from the respiratory tract.

No attempt was made in these experiments to separate the water vaporized from the respiratory tract from that vaporized from the skin. In two experiments, however, calculation was made of the possible maximum increase in water loss from the lungs upon the assumption that the expired air was saturated with water vapor at body temperature. The following results were obtained. In experiment 4, 32.3 grams more water per square meter per hour were lost than normally during the third period following cocaine. Calculation shows that only 8.7 per cent of this increase could be due to increased vaporization from the respiratory tract. In experiment 5, there was an increase of 48.2 grams per square meter per hour in total water loss above the average normal during the second period following cocaine. Similar calculation shows that not over twenty-two per cent of this increase could be due to increased vaporization from the respiratory tract.

The above examples show therefore that the increase in volume of expired air plays little part in the increased water loss even if the assumption that the expired air is completely saturated is correct. Granting that expired air is normally completely saturated it is evident that the air collected under the conditions of these experiments is not completely saturated, due to the extra "dead space" in the mask and T-tube. The inspired air is probably a little more saturated than the relative humidity figures show, due to the contact of the in-going air with the moist valves and T-tube. Either of these factors would tend to lower the percentage of water loss attributable to the expired air. It is evident then that most of the increase in water loss in the cocaine poisoned rabbit must be attributed to increased vaporization from the skin.

DuBois' (1924) metabolic studies of the malarial chill and the intravenous injection of foreign protein as well as of typhoid bacilli in man, have considerably elucidated this problem of the onset of fever. "Under ordinary conditions of health," says DuBois, ". . . . extra calories would be eliminated through the skin. But some coöperative mechanism prevents this during a chill." He pictures the zone where the blood is cooled as being withdrawn to a deeper level some distance below the surface of the body, thus converting the integument to a mere suit of clothes. How such a change affects radiation is well recognized, but DuBois has added to our knowledge of the process in the three types of fever mentioned, a picture of the behavior of the vaporization calories. While he found these often increased a few per cent during the crucial chill period, such increase is very insignificant in proportion to the enormous rise in the heat production, which was often more than doubled. Following the chill period which consumed thirty to forty minutes, the vaporization calories again assumed a more significant proportion of the total calories, the febrile temperature plateau having been attained. What has just been said of the vaporization calories applies essentially to DuBois' data on the total heat loss.

We have now demonstrated a precisely similar sequence of events in rabbits, and have been able to account for the same quantitatively by our findings on liver hydration. The chief significance of our work seems to lie in developing the conception that while fever is taking hold of the body, the liver is grasping water. This seems to keep down the hydration of the blood sufficiently to prevent the physiologic increase in surface evaporation which should accompany extra heat production. But the interference is only temporary; after a half-hour or so events take a new turn. The liver now passes on its stored water for use in the maintenance of heat elimination at a higher level.

SUMMARY AND CONCLUSIONS

The insensible weight loss was followed in five fasting rabbits given cocaine hydrochlorid in fever-producing doses (twenty to thirty milligrams per kilo). In four of the rabbits the gaseous metabolism was determined. Water loss was found practically identical with total insensible loss until the fever was well advanced.

During the first half-hour after cocaine injection there was a definite fall in the water loss in two rabbits, a slight rise in a third and a marked increase in the other two (which showed the greatest increases in the total metabolism). The ratio of vaporization calories to total calories was reduced in all cases except one, in which there was no change. Beginning with the second half-hour or hour of fever, a marked increase in the vaporization : metabolism ratio was observed.

The delay in the rise of the vaporization : metabolism ratio corresponds in time to the period of maximum liver hydration; evidently the ratio begins to rise as the excess water leaves the liver again. In an average case a two kilo rabbit with cocaine fever loses an excess of 3 or 4 cc. per hour by evaporation, while the liver is yielding its excess water at the same rate.

The retention of water by the liver in the first stage of fever seems to play a leading rôle in the interference with heat elimination which causes the febrile temperature.

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SOME FACTORS REGULATING THE UTILIZATION OF SPLENIC IRON

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The results of numerous investigations suggest that there is present in the spleen and liver of mammals a store of iron which may be utilized for the formation of hemoglobin (Asher, 1908; Muir and Dunn, 1915; Whipple and Robscheit-Robbins, 1925; Jencks, 1922). In the former organ at least the reserve iron is preponderantly, if not exclusively, present in the form of hemosiderin, the nature of which has been investigated by one of us (Cook, 1928), and reported to be probably inorganic, colloidal iron. Furthermore, of the total splenic iron content by far the greater portion is in the form of hemosiderin. Such being the case, it is possible to investigate further the rôle of hemosiderin using the total splenic iron as an index to the hemosiderin, or reserve iron, content.

From the point of view of the availability of the iron reserve, various investigators (Williamson and Ets, 1927; Williamson and Ewing, 1928) have made studies on the livers and spleens of dogs and rats. They have found that in several cases the administration of small quantities of ferric salts, orally or subcutaneously, do not increase the amount of reserve and do not bring about hemoglobin regeneration in anemic animals. But "food iron" results in the storage of that element in a form available for subsequent emergency, such as hemorrhage.

On the other hand, several workers have demonstrated beyond much doubt, the activity of copper salts in the building of hemoglobin (Hart, Steenbock and Elvehjem, 1925; Hart, Steenbock, Elvehjem and Waddell, 1928; Waddell, Steenbock, and Hart, 1929a; Waddell, Steenbock, Elvehjem and Hart, 1929; Waddell, Steenbock and Hart, 1929b; Beard and Myers, 1929; McHargue, Healey and Hill, 1928). The efficacy of copper in the remission of experimental anemia and in the therapy of pernicious anemia suggests the possibility that this element is concerned with the utilization of that iron which is stored as hemosiderin. This investigation was undertaken for the purpose of ascertaining whether copper, as administered in the diet, has any effect on the mobilization of the splenic iron reserve. In addition to the possible rôle of copper, the age of the animal, especially in

the younger stages, appears to be of significance in regard to the utilization of splenic iron.

METHODS. For these studies young rats were used because they were readily available and easily kept. The animals were obtained from a number of sources; most of them were from the departments of Zoology and Anatomy of the University of California, a few were available from the stock of the Physiology Department, and one litter were unwanted pets. Since the diet of the mother during pregnancy would affect the amount of iron reserve in the young it may be well to mention the diet of the parents of the animals used. The departments of Anatomy and Physiology feed a diet (Lepkovsky and Singer) which is composed of fish meal, whole wheat, whole milk powder, yeast, sodium chloride and calcium carbonate. Ten per cent fish oil is added to insure an adequate source of vitamins A and D. An analysis of this diet was made to determine the amount of iron in a day's ration. Fourteen and a half grams of the mixture contained approximately one milligram of iron, which should be sufficient even during gestation. The rats procured from the Zoology department had much the same nutritional background. The maintenance diet consisted of ground wheat, alfalfa meal, fish meal, fish oil, casein, and sodium chloride. Fresh milk was added to the feed of the females during pregnancy and lactation. Lettuce was given twice a week. The owner of the pets used reports that the adults were given table scraps.

The usual procedure was to take the young from the mother on the twenty-first day and immediately put them on an iron-low diet to which various supplements were added. To the basic diets, consisting of rice and milk, or of milk alone, a drop of cod liver oil per rat per day was added. The following variations were studied.[1]

1. Rice, milk, sodium chloride, calcium carbonate.
2. Rice, milk, salts as above, Eli Lilly's liver extract, no. 341 (0.3 gram per rat per diem, the equivalent of 0.0075 mgm. copper).
3. Rice, milk, salts as in no. 1, copper sulphate (0.05 mgm. copper each per diem).
4. Rice, milk, salts as in no. 1, beef spleen ad lib.
5. Milk + 0.0075 mgm. copper, as sulphate, daily.[2]

[1] In the case of diets 2, 3, 5, 6, and 7 complete consumption of the supplement was insured by giving it in a very small quantity of milk in the first feeding.

[2] Two recent investigations have been reported in which copper was fed to mammals in larger doses than we have found advisable. Hall and Mackay (1930) fed rabbits 2 mgm. copper per gram of food per day. Keil and Nelson (1931) fed rats 3 mgm. copper daily in one set of experiments and 0.05 mgm. copper daily in another. The mortality reported by the former investigators, and also that observed by ourselves with doses of as much as 0.05 mgm. copper per rat per day emphasizes the necessity for caution in the administration of copper lest the beneficial effect of minute quantities of the element be vitiated by the toxic action of too great quantities.

6. Milk + 0.5 mgm. iron, as citrate, daily.

7. Milk + 0.0075 mgm. copper and 0.5 mgm. iron, as sulphate and citrate respectively.

8. Bran, rolled oats, milk powder, lettuce, sodium chloride, and calcium carbonate.

According to the tables of Peterson and Elvehjem (1928) and those of Lindlow et al. (1931), diet 8 is fairly high in iron and contains considerable copper.

Unless otherwise noted, the diets were continued for six weeks, at the end of which time the rats were killed with ether. This method of killing insured contraction of the spleen, with consequent expulsion of practically all the blood. Any significant amount of reservoir blood might have invalidated the analyses for iron.

The spleens were removed immediately, weighed and dried. They were then digested with concentrated hydrochloric acid, which was subsequently evaporated. (Digestion in this manner prevented the troublesome foaming which occurs when spleen tissue is heated.) The residues from this treatment were ashed and the ash dissolved in a few cubic centimeters of concentrated hydrochloric acid. The resulting solution was oxidized by treatment with concentrated sulphuric acid, and the hydrochloric acid boiled off. Complete conversion of the iron from the ferrous to ferric state was insured by the addition of a small quantity of potassium permanganate solution. The analysis was by titration with titanous chloride solution ($TiCl_3$) according to the method outlined by Knecht and Hibbard (1925). Potassium thiocyanate was used as an indicator. Although the $TiCl_3$ solution was kept under hydrogen, some oxidation to the tetrachloride slowly took place. For this reason standardization of the reagent was performed before each set of analyses by titrating against standard iron solution (ferrous ammonium sulphate).

In all cases the amount of iron found is expressed as milligrams per gram of tissue.

Results. I. A group of rats kept on a diet of rice and milk was used for a study of the relation of age to the amount of splenic iron. They were not invariably litter mates but their ages varied less than a week. By sacrificing members of this group at intervals, we divided them into four sub-groups.

The results of the analysis of the spleens of these four sub-groups of varying ages (see table 1) indicates a gradual withdrawal of the iron from the spleen as the animal grows.

From the statistical means of these four age control groups and of another group of much older rats the decrease in the splenic iron is seen to be more rapid in the period immediately after birth, the rate of withdrawal gradually becoming less. The results suggest that a logarithmic relationship

might exist between the values of the iron reserve and the age, but the data are insufficient to warrant any rigid mathematical treatment.

II. An analysis of the relation of body weight, weight of the spleen, absolute amount of iron present and milligrams of iron per gram of spleen was then made.

Table 2 gives the data for twenty-five rats of the age of nine weeks which had been kept on an iron-low diet for six weeks. From these data the coefficients of correlation were calculated according to the method of Galton as described by Sinnot and Dunn (1925)[3] (see table 3).

TABLE 1

Variation of splenic iron with age

AGE	NUMBER OF RATS	MGM. Fe / GRAMS SPLEEN STATISTICAL MEAN	REMARKS
1 day	4	2.10	One litter, killed on day of birth. Because of small size all four spleens were analyzed together
4–5 weeks	3	1.17	
6–7 weeks	4	0.46	
9 weeks	4	0.42	
12–16 weeks	4	0.20	Young males. Diet varying. Included for comparison only

The coefficient of correlation between the weight of the animal and the weight of the spleen is 0.52 ± 0.098. Due to the small number of cases studied, the probable error is more than one-sixth the value for the coefficient of correlation; there is roughly a 50 per cent correlation between body weight and weight of the spleen. There is no correlation between body weight and the amount of iron in the spleen. The coefficient of correlation between the body weight and the number of milligrams of iron per gram of spleen is very high; $r = -0.82 - 0.44$. This indicates that in the case of young rats, the more rapid the growth, the greater is the strain put on the iron reserve. This finding is in accord with the observations that rapidly

[3] The formula used is:

$$r = \frac{\frac{\Sigma f}{n} - (Cx \times Cy)}{\sigma x \times \sigma y}$$

Where Σf represents the summation of frequencies, $(Cx \times Cy)$ the product of the corrections, σ the standard deviation, and n the total number of cases, here 25. The probable error, Er, is calculated according to the following formula, where r and n have the same significance:

$$Er = \frac{0.6745\,(1 - r^2)}{n}$$

growing animals develop nutritional anemia sooner than those which grow more slowly (Nevens and Shaw, 1930).

The weight of the spleen is only slightly related to the amount of iron present, as shown by a coefficient of correlation of 0.48 ± 0.1. Likewise, the relation between the weight of the spleen and the proportion of iron present is low— -0.52 ± 0.098. There is no correlation between the milli-

TABLE 2

Data for twenty-five rats fed on iron-low, copper-low diets showing body weight, spleen weight, total iron content, and relative iron content, the latter expressed as $\frac{mgm.\ Fe}{gram\ spleen}$

BODY WEIGHT	WEIGHT OF SPLEEN	Fe PRESENT	MGM. Fe/GRAM SPLEEN
grams	*grams*	*mgm.*	
140	0.407	0.13	0.32
128	0.378	0.15	0.39
128	0.269	0.15	0.56
116	0.595	0.11	0.12
100	0.204	0.08	0.41
97	0.709	0.29	0.40
94	0.254	0.08	0.40
88	0.151	0.05	0.29
84	0.442	0.10	0.22
80	0.263	0.08	0.28
80	0.143	0.10	0.70
72	0.158	0.05	0.30
70	0.054	0.06	1.10
70	0.266	0.10	0.35
65	0.135	0.08	0.55
64	0.155	0.06	0.38
63	0.135	0.03	0.23
62	0.324	0.06	0.20
60	0.133	0.13	0.91
60	0.084	0.06	0.71
58	0.066	0.05	0.68
48	0.135	0.08	0.55
47	0.087	0.14	1.61
44	0.083	0.10	1.02
36	0.187	0.10	0.53

grams of iron actually present in the spleen and the milligrams of iron per gram of spleen as might be deduced from the above correlations.

This statistical treatment shows no outstanding relationships except in the case of body weight and milligrams of iron per gram of splenic tissue This value (-0.82 ± 0.044) is large enough to show a close connection between the two values. The larger the animal, the more blood it is necessary for it to produce; therefore when the dietary iron is low, the withdrawal

of iron from the reserve stores is greater in the larger and more rapidly growing animals than in small ones.

III. The investigation of the effect of metal salts was based upon four general types of diet: 1, iron-low copper-low; 2, iron-low copper-high; 3, iron-high copper-low; and 4, iron-high copper-high. The composition of these, with their variations, has been described in detail in a preceding section. Unless otherwise noted several rats were examined (3 to 5) with each variation of diet. The results are recorded in tabular form (see table 4).

From the data presented in table 4 it appears that the iron reserve of animals which received copper and inadequate iron tends to be definitely lower than the reserve of those rats fed on a diet very low in both iron and

TABLE 3

Coefficients of correlation between the items listed in table 2: Body weight, spleen weight, total, and relative iron content

BODY WEIGHT	WEIGHT OF SPLEEN	Fe PRESENT
		mgm.
1. Correlation with weight of spleen: $r = 0.52 \pm 0.098$	1. Correlation with total iron content of spleen: $r = 0.48 \pm 0.1$	1. Correlation with relative iron content of spleen: $r = 0.01$ (negligible)
2. With total iron content of spleen: $r = 0.0012$	2. With relative iron content of spleen: $r = 0.52 \pm 0.098$	
3. With relative iron content of spleen: $r = -0.82 \pm 0.044$		

copper. The mean for all the animals receiving iron-low, copper-low diets for six weeks is 0.54 $\frac{\text{mgm. Fe}}{\text{gram spleen}}$ as contrasted with approximately half that amount for the animals receiving a supplement of copper. The addition of considerable iron to the diet (in different forms), the copper being kept low, did not appreciably increase the splenic iron (cf. Williamson and Ets, 1927).

In decided contrast is the behavior of the iron reserve in those cases where considerable iron and copper were combined in the diets. Here in general the iron reserve is much higher, depending roughly on the amount of iron fed.

1. When the metals are fed as they occur in Lilly's Liver Extract the copper is high (0.0075 mgm. per rat per day). The extract was shown by analysis to contain approximately 0.25 mgm. iron per gram. Such a diet can not be said to be iron-low in the sense that milk and rice diets are iron-

TABLE 4

Effect of metals in the diet on splenic iron

Each rat was fed the diet indicated from the third to the ninth week, at the end of which period it was killed.

NUMBER OF RATS	TYPE OF DIET	AVERAGE AMOUNT OF IRON: $\frac{\text{MGM. Fe}}{\text{GRAMS SPLEEN}}$	REMARKS
4	Iron—low Copper—low	0.42	Anatomy stock. Diet: rice and milk
4	Iron—low Copper—low	0.47	Pets. Same diet
3	Iron—low Copper—low	0.53	Anatomy stock. Same diet
3	Iron—high Copper—low	0.45	Anatomy stock. Iron as ground beef spleen
1	Iron—high Copper—low	0.47	Zoology stock. Iron as ferric citrate (0.5 mgm. daily)
1	Iron—low Copper—high	0.19	Zoology stock. Copper as $CuSO_4$ (0.05 mgm. daily per rat). Sole survivor of a group of twelve. Mortality probably due to toxicity of copper
4	Iron—low Copper—high	0.30	Zoology stock. Copper as $CuSO_4$ (0.0075 mgm. daily per rat)
2	Iron—low Copper—high	Trace	Zoology stock. Iron reserve of this litter abnormally low. Two controls fed on copper-low diet showed iron content of 0.074 $\frac{\text{Mgm. Fe}}{\text{gram spleen}}$
3	Iron—high Copper—high	0.41	Anatomy stock. Iron as Eli Lilly's liver extract. The extract also contains copper (0.0075 mgm. Cu per rat per day)
2	Iron—high Copper—high	0.41	Anatomy stock. Same diet. A different litter
2	Iron—high Copper—high	0.83	Zoology stock. Iron as ferric citrate. Copper as $CuSO_4$
5	Iron—high Copper—high	1.46	Zoology stock. Iron and copper as "food metal." Given as diet no. 8

low because with the extract there is an appreciable amount of iron, whereas with the true iron-low diets there is barely a trace of the element. The rat is apparently able to utilize quantities of this magnitude (about 0.08 mgm. daily) in the presence of adequate copper and thus maintain its iron reserve.

2. More iron was fed when ferric citrate was added to the basic diet and here the reserve nearly doubles.

3. When the metals are fed as "food iron" and "food copper" the value is approximately three times as great as under iron-low, copper-low conditions. The greater effect in this case may possibly be due to the mode of combination of the iron (presumably organic) but it is quite likely that the absolute amount was greater than in the preceding case.

On the latter assumption, if the copper content of the diet is high, the amount of iron in the reserve is more or less proportional to the amount of iron fed. Summarizing from table 4 we see that if the iron content of the diet is increased from a trace in the low iron diet to a maximum in the high "food iron" diet that the mean values of the reserve increase thus: 0.3—0.4—0.83—1.46.

Discussion. The results of this investigation suggest that when copper is added to a diet deficient in iron, a mobilization of the iron reserve (at least of the splenic iron) takes place. This activity of copper suggests a possible explanation for the results of McHargue et al., (1928) who found hemoglobin regeneration in anemic rats fed milk supplemented by copper salts.

Titus and Hughes (1929) have shown that copper is stored in the animal body to a slight extent. We might suggest that the "carry-over" period or delay in the development of anemia, noted by Whipple and Robscheit-Robbins (1925) and Jencks (1922) is due not only to the available reserve of iron, but to the presence of this small store of copper and possibly other accessory elements. In this connection, it is interesting to note that the livers of very young animals contain more copper than the livers of adults (Lindlow et al., 1929). The findings here reported indicate that the depletion of the iron reserve is most rapid in the first weeks after birth when the copper reserve is still high.

From this evidence we may conclude that an important rôle of copper is the facilitation of the transformation of hemosiderin into a form which can be utilized by the body, thus making the iron reserve available for hemoglobin building. Hence the administration of copper salts may prove efficacious in the remission of anemia, whereas iron salts of high purity cannot be utilized (cf. Keil and Nelson, 1931).

Iron, fed in the complete absence of copper, is not available for the formation of hemoglobin and the withdrawal of splenic iron proceeds in practically the same manner as in the case of animals fed on a diet deficient in

both copper and iron. The values of the splenic iron are practically the same for animals on the basic diet and those which received an iron supplement. But when adequate copper is added to a diet in conjunction with iron, the amount of iron in the spleen is approximately proportional to the diet, and the result is usually an increase in splenic iron over the amount found during administration of the basic copper-poor diet. A possible explanation for these high values in the case of rats fed both iron and copper is the following. When iron is fed with copper, the former element is capable of direct utilization in hemoglobin formation. Hence there is no necessity for drawing on the splenic iron reserve. The normal destruction of red blood cells, however, leads to a continuous accumulation of iron as hemosiderin in the spleen. Therefore the level of the splenic iron reserve at the end of the experiment is found to be higher than in the case of any other combination of dietary iron and copper.

SUMMARY

The relative amount of iron in the spleen decreases with age, the rate of the withdrawal decreasing with age.

The proportion of iron to the weight of the spleen bears a negative relation to the body weight, the coefficient of correlation of these two values being -0.82 ± 0.045.

The addition of small amounts of copper to an iron-low diet results in greater depletion of the reserve iron of the spleen.

When copper is added to a dietary supplied with sufficient iron, the amount of splenic iron is greatly increased, while iron alone does not produce an augmentation of the splenic reserve. An explanation for this phenomenon is offered.

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BLOOD REGENERATION STUDIES

I. Changes in the Volume, Number and Size of the Erythrocytes in Hemorrhagic Anemia[1]

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Evidence has been accumulating in recent years in support of the contention that alteration in the size of the red corpuscles is of diagnostic value in helping to differentiate the various types of anemias. That the red blood cells are increased in diameter in pernicious anemia has been well established by the work of Price-Jones (1922), Bell, Thomas, and Means (1926) Medearis and Minot (1927), Persons (1929) Fitzhugh and Persons (1929) and others. Studies which have been reported on the alteration in the size of the red blood cells in secondary anemia have been less in agreement. Price-Jones (1910) found that the average red cell diameter of a rabbit which has been subjected to moderately severe hemorrhage increased synchronous with the formation of large nucleated red cells in the marrow. In 1922 in a clinical study of 10 cases of hemorrhagic anemia, he observed a decrease in the mean diameter of the erythrocytes. Jolly (1923) working with dogs, and Ponder and Miller (1928) experimenting with rabbits reported increases in the diameter of red cells following hemorrhage. The volume of the individual red cells has also been studied in this connection. Haden (1923) reported that in chronic hemorrhagic anemia, the red cells had a smaller volume than normal. Murphy and Fitzhugh (1930) observed increases in the average individual cell volume in patients following acute hemorrhage, whereas in anemia resulting from chronic loss of blood, the individual cell volume was generally low. Mayerson and Laurens (1931) suggested that the decreased size of cells which they observed was due not only to the removal of cells of normal size by the hemorrhage, but also to the unfavorable conditions of oxygen want due to the absence of any increase in hemoglobin production.

Because secondary anemia is of common occurrence, and because there is considerable disagreement in the published data on the blood picture in this condition, it seemed that a study of the diameter and volume of the

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red cells in relation to total cell volume and cell count would be of value. Any consistent and significant departures from the normal range should be useful in helping to diagnose this type of anemia

EXPERIMENTAL. The experimental animals used in this study were seven normal adult dogs, two females and five males, ranging in weight from 16½ to 22½ kgm. One of the animals was on a diet of table scraps (dog 14) throughout the experiment and the other six were on a synthetic diet. This diet, which was based on that of Karr (1920), had the following composition:

Casein	6.3
Sucrose	4.5
Cod liver oil	0.6
Lard	3.3
Bone ash	0.25
Salt mixture	0.2
Dried yeast (baker's)	0.5
	15.65

The salt mixture was that devised by Karr (1920) with the addition of 0.2 gram copper sulfate to the formula. The yeast in the diet of 3 of the dogs had been autoclaved to destroy the antineuritic vitamin using the procedure as outlined by Hogan and Hunter (1928). The diets were fed at a level which would keep the animals at a fairly constant body weight.

After the animals had been in the laboratory sufficiently long to become accustomed to the diet, laboratory conditions, and routine, blood samples were taken to obtain some normal figures. Total blood volume was determined according to the procedure outlined by Hooper, Smith, Belt and Whipple (1920). For the red cell counts and cell diameter determinations, two separate dilutions of the red corpuscles in Hayem's solution were made, shaken 3 minutes, and a drop of each spread on a Levy-Hauser counting chamber. The cells lying in 100 consecutive squares were counted and then measurements of the red cell diameters were made on the same slide. To insure greater accuracy in measuring, a no. 1-A cover slip was used. Measurements of 1 diameter of 50 red blood cells were made by means of a calibrated Bausch and Lomb filar micrometer using a magnification of 560 diameters. Readings were made to 0.2 micron.

Since only 1 diameter was measured, it was, of course, necessary to select only round cells for measurement. It is recognized that this introduces a certain degree of selectivity into the measurements and therefore also a certain degree of inaccuracy. It would seem, however, that the error probably works in both directions and therefore tends to neutralize itself. In order to determine the extent of error in sampling as a result of measuring only 50 cells, duplicate sets of 50 cells each were measured on the same

slide in a series of 10 consecutive slides. The maximum difference observed was 0.14 micron; the average difference was 0.07 micron. From this it was concluded that a measurement of 50 cells gave sufficient accuracy for the purposes of this experiment.

Individual red cell volume was calculated using the formula of Murphy and Fitzhugh (1930) which is as follows:

$$\frac{\%\text{ of cells by hematocrit}}{\text{No. red cells in 100 cc. blood}} = \text{individual cell volume } (\times 10^{-11}\text{ cc.})$$

Anemia was induced by 2 large hemorrhages spaced 2 or 3 days apart, one-fourth of the determined total blood volume being removed from the

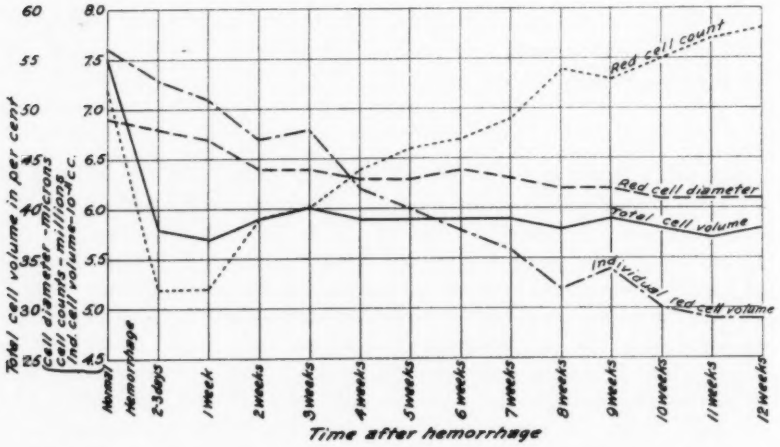

Fig. 1. Changes occurring in cell count, cell diameter, and cell volume in hemorrhagic anemia.

jugular vein on each occasion. Following the second large bleeding, blood samples amounting to approximately 50 cc. were removed on alternate days for the first week, and thereafter once weekly for a period of 12 weeks. This amount was required to supply enough blood for the determination of total blood volume, red cell hematocrit, hemoglobin, red and white cell counts, total nitrogen, non-protein nitrogen, urea, creatinine, and amino acid nitrogen. The results of these studies will appear later.

The seven animals in this experiment were in good health at the beginning of the study and continued so throughout, although the animals on the synthetic diet low in vitamin B showed loss of appetite after they had been on the diet a number of weeks. This was readily relieved, however, by the administration of dried whole yeast in 10 gram doses on three consecutive

days. Since diet did not appear to influence the results in this study, it was considered justifiable to average the data secured on this group of animals.

DISCUSSION. *Cell volume per cent.* The average cell volume in per cent for the 7 animals as determined before bleeding and for the 12 weeks following bleeding is shown in figure 1. The average cell volume for the group before bleeding was 54.9 per cent, the range being 46.1 to 61.8 per cent. The first determination following hemorrhage showed that the corpuscle volume had decreased as a result of the removal of approximately one-half of the total determined blood volume to an average of 38.2 per cent for the group, a decrease of about 30 per cent. It is also apparent that cell volume per cent was practically unchanged throughout the entire post-bleeding period, the average for the group at the end of the experiment being, it so happens, identical with the first determination made following the second large hemorrhage. The 50 cc. sample which was removed each week no doubt was a factor in preventing a return to the normal level.

Red cell count. The erythrocyte count before bleeding averaged for the group 7.22 million per cmm., the range being from 6.43 to 7.89 million per cmm. Following bleeding, the average for the group was 5.24 million per cmm., a decrease of approximately 27 per cent. Throughout the regeneration period, there was a steady increase in cell count, the most rapid increase in cells occurring between the 1st and 2nd week following bleeding. As far as cell count was concerned, recovery was complete before the end of the experiment, since the average for the group after the 8th week was actually higher than the pre-bleeding normal.

Red cell diameter. The normal pre-bleeding average red cell diameter for the group was 6.9 microns, the range being 6.8 to 7.0 microns. As shown in figure 1 there was a gradual decrease in red cell diameter, beginning as early as the first determination following hemorrhage, when the average observed was 6.8 microns. At the end of the 12th week, the average for the group was 6.1 microns, the range at this time being 5.9 to 6.4 microns. There was a marked decrease in cell diameter in all of the animals, the decrease ranging from 0.5 to 1.0 micron. The most marked change occurred between the 1st and 2nd week. This, it will be recalled, was also the period when the most rapid increase in the number of cells was observed.

Dog 14 was continued in the laboratory for 8 more weeks during which time blood samples amounting to 35 cc. were removed each week. A determination made at the end of the 20th week showed red cell diameter 5.9 microns, cell count, 9.26 million, and cell volume 49.2 per cent. This indicated that cell volume and cell diameter were still considerably below the pre-bleeding level for this animal, whereas the cell count was much above the pre-bleeding level. As a matter of fact, cell diameter had decreased considerably since the 12th week of the post-bleeding period.

In order to determine whether or not the decrease in cell diameter was due to the production of a large number of cells of very small size which brought down the average or to a decrease in the diameter of the cell population as a whole, a study was made of the spread or scatter in red cell diameter throughout the experimental period. The cells in the normal pre-bleeding blood samples ranged in diameter from 5.5 to 8.4 microns, 97 per cent falling within the range 6.0 to 7.9, and approximately 50 per cent within the very narrow range of 6.5 to 6.9 with 28 per cent greater and the balance smaller than this size. The result of hemorrhage was to produce a shift in the cell population toward the smaller size, the extent of the shift increasing as the experiment continued. There was a steady decrease in the proportion of large cells and an increase in the proportion of small cells. This was apparent from a comparison of the range during any week of the post-bleeding period with the pre-bleeding normal. During the sixth week of the post-bleeding period, for example, the cells ranged in size from 4.5 to 7.9 microns. At this time the range from 6.5 to 6.9, which in the normal figures constituted approximately 50 per cent of the total, now constituted only 38 per cent with only 9 per cent larger as compared with 28 per cent in the normal blood, and 53 per cent smaller, as compared with 22 per cent in the normal. At the termination of the experiment the cells ranged in diameter from 3.5 to 7.9 microns. Again comparing this with the normal, we find that only 24 per cent are now found in the 6.6 to 6.9 group, with $3\frac{1}{2}$ per cent larger and $72\frac{1}{2}$ per cent smaller. Furthermore, the group 5.5 to 5.9, which included the smallest cells observed in the normal blood and which constituted only 2.6 per cent, at the end of the experiment was actually the largest group, constituting over 35 per cent. Although it is quite possible that small numbers of cells of normal size were constantly being formed, it would seem that the time interval during which the cells of normal size were almost completely replaced by cells of much smaller size offers some information as to the length of life of the red blood cell.

Figure 2 illustrates this alteration in the size of the cells. Whereas the normal curve is fairly symmetrical and has a narrow spread, the effect of hemorrhagic anemia is not only to increase the spread but also to produce an asymmetrical curve. This is in agreement with the observations of Price-Jones (1922) who reported that the curves for pernicious anemia lie to the right and the curves for hemorrhagic anemia to the left of the normal curve. He states that the curve for the normal cells is more or less symmetrical, whereas those for pernicious anemia and hemorrhagic anemia are asymmetrical in type.

Individual red cell volume. The average normal individual cell volume for the group was 7.62×10^{-11} cc. As may be seen in figure 1 there was a steady decrease in the individual cell volume throughout the experiment,

beginning as early as the first determination following bleeding. The average individual cell volume at the end of the experiment was 4.9×10^{-11} cc. which was nearly 36 per cent less than the normal volume. This decrease in individual cell volume was to be expected in view of the decrease in cell diameter which was observed. The decrease in volume, however, appears to be more pronounced than the decrease in diameter. Haden suggests that the extent of the decrease in cell volume is probably the best index of the drain on the bone marrow and is of definite prognostic value.

Correlations which were run between the several factors studied indicated a definite relationship in each instance. The coefficient of correlation between cell volume and cell diameter was $+0.8230 \pm 0.0220$, indi-

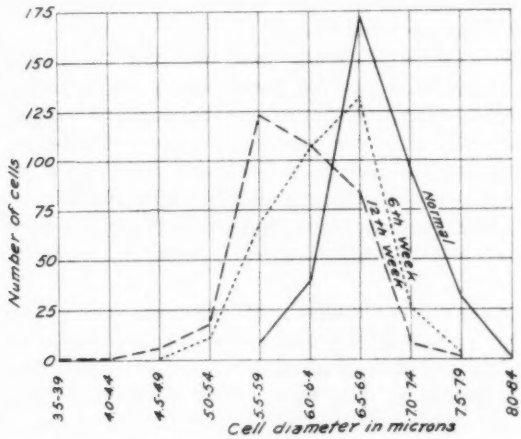

Fig. 2. Cell distribution in dog's blood during the sixth and twelfth week following the removal of approximately 50 per cent of the total blood volume, compared with the pre-bleeding normal cell distribution of the same animals. Each curve is based on the measurement of 350 cells.

cating that cell diameter is a satisfactory measure of cell volume. The coefficient of correlation between cell count and cell diameter was -0.4666 ± 0.0533 and between cell count and individual cell volume, -0.5856 ± 0.0448. The fairly high negative correlation observed in both these instances indicates the close relationship which exists between the size of the cell and the number which are formed in hemorrhagic anemia. Periods of rapid cell formation are associated with a marked decrease in cell size. The determination of either cell diameter or individual cell volume would seem to be desirable in following a case of hemorrhagic anemia.

The diets used in this study apparently were not very favorable for the formation of red blood cells. Since dog 14, on the diet of table scraps,

showed no better recovery than did the other animals in the study, it would seem that the synthetic diet was as satisfactory for blood regeneration as was a diet of table scraps. Furthermore, since this dog was the largest in the group the weekly removal of 50 cc. of blood should have proven less of a physiological strain than in the case of the smaller animals. In the present study the amount of reserve material for red cell formation appeared to be the factor of first importance in determining the extent of recovery.

SUMMARY

1. Seven dogs rendered anemic by the removal of approximately one half of their determined total blood volume were observed for a period of twelve weeks following hemorrhage.

2. The diets used in this study were not very satisfactory for blood regeneration, although as good results were obtained with the animal on a diet of table scraps as with the animals on the synthetic diet.

3. Total cell volume in per cent was unaltered throughout the entire post-bleeding period; the average for the group at the end of the experiment was identical with the first determination following bleeding.

4. Red cell count had returned to normal by the eighth week of the post-bleeding period, and thereafter increased considerably above the normal pre-bleeding level.

5. The diameter of the red blood cells decreased during the regeneration period from an average of 6.9 microns before bleeding to an average of 6.1 microns at the end of the post-bleeding period. One animal which was studied for an additional eight week period showed a continued decrease in cell diameter during this period, although cell volume per cent approached the normal and red cell count was much above the normal at the end of this time.

6. Throughout the post-bleeding period there was a steady decrease in the proportion of normal size cells and an increase in the proportion of cells of much smaller size. Curves illustrating the scatter in cell diameter show that the normal curve is fairly symmetrical and has a narrow spread. The curve for hemorrhagic anemia lies to the left of the normal, has a wider spread, and is asymmetrical in type.

7. Calculation of the individual red cell volume showed a steady diminution in cell volume which was synchronous with the decrease in cell diameter. The coefficient of correlation between cell diameter and cell volume was $+ 0.8230 \pm 0.0220$.

8. A rapid increase in the number of red cells was associated with a marked decrease in cell diameter and individual cell volume. The coefficient of correlation for cell count and cell diameter was $- 0.4666 \pm 0.0533$ and for cell count and cell volume, -0.5856 ± 0.0448.

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COMPARATIVE STUDIES ON THE PERIPHERAL AND CENTRAL RETINA

IV. Temporal Summation of Subliminal Visual Stimuli and the Time Course of the Excitatory After-Effect

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Bloch (1885) discovered that for short flashes of light the product of intensity, i, and time of exposure, t, are constant at the threshold and the observation has since been repeatedly confirmed. Although the simple formula $it = c$ seems to be a fairly good approximation for times between 2 and about 30σ (Piéron, 1920a, b; Blondel and Rey, 1912; and Reeves, 1918), for longer times of exposure the relation may assume the form $it = a + bt$ (Blondel and Rey). Braunstein (1923) found $it = c$: only within about 10σ and only with small areas. For very short times the relation breaks down. The obvious analogy between Bloch's rule and the Bunsen-Roscoe law, according to which the effect in photochemical reactions is directly proportional to the product of time of exposure and light absorbed, has led to interesting attempts to correlate the two particularly by Hecht, although as that author has pointed out (1929) there is evidence to show that such correlations in so far as the vertebrate retina is concerned are necessarily somewhat schematic.

Adrian and Matthews (1927) working on the excised eel's eye and using the latent period of the discharge in the optic nerve as an index of the intensity of the physiological process found that with supraliminal stimuli the product $i \times t$ was integrated just as in the experiments on the eye reported above. In a later paper (1928) they were also able to show that this index, the latent period, was influenced by synaptic processes in the retina. It was, as a matter of fact, by measuring the latent period that they discovered spatial summation between separated stimuli and proved that the interchangeability between area and intensity was due mainly, if not altogether, to synaptic interaction. In the light of this finding, it is now possible to understand the fact which they pointed out in their second paper, that area, a, stimulated also influenced the result so that approximately corresponding latencies were given by equal energies, defined

as equal products of $i \times t \times a$.[1] It would seem therefore as if part at least of the integration were synaptic in origin and the latent period were partly, or perhaps mainly, the time taken by the stimulus to attain neurone threshold. (See the recent important work of Eccles and Sherrington, 1931; Eccles, 1931.) In this connection it is interesting to note that Piéron has found in the human eye that the smallest quantities of energy necessary for threshold stimuli are obtained with relatively large areas stimulated for short times (about 10σ).

Previous papers in this series (Granit, 1930; Granit and Harper, 1930) show that with supraliminal stimuli spatial summation between separated areas occurs also in the human eye with sensory phenomena as indicators. In the present paper our main problem is to determine whether there is evidence for synaptic summation of two *successive* subliminal stimuli. It is evident that the reflex concept of temporal summation implies that, to use Sherrington's words, mere duration of a stimulus becomes equivalent to intensity ("addition latente," Sherrington, 1924–25). A relation of this general type is Bloch's rule, as well as the formula given by Blondel and Rey. From Richet and Bréguet's (1880) and Rutenburg's (1914) work we know that subliminals actually do sum in the human eye.

METHOD. Our method is taken from experiments on summation of successive subliminal stimuli in the spinal flexion reflex by Eccles and Sherrington (1930) which have been modified to fit the demands of a visual problem. The principle underlying this method is the use of a second subliminal as a test of the after-effect left by a subliminal predecessor. Eccles and Sherrington sent two electric shocks at various measurable intervals into two nerves of the limb and recorded isometrically the amount of reflex contraction elicited by the second shock in consequence of its acting upon the after-effect of the first. As a matter of course neither of them caused any contraction alone. As used for the present work in vision it means that a second subliminal flash will have to be adjusted so as just to be able to raise the after-effect of a preceding invisible flash above the threshold. It is then evident that the intensity necessary for the second subliminal, with various intervals between the two, must depend upon the amount of excitatory remainder left by the first. The higher the level of the excitatory after-effect, the less has to be added to it in order to make it visible. In this way it should be possible to map out the time course of a subliminal excitatory state just as was done by

[1] Adrian and Matthews did not rediscuss their earlier findings in the light of the important results brought forth in their third paper, which definitely excluded certain alternative explanations suggested in the discussion attached to the second paper. This probably explains why their proof that synaptic factors are involved in the latent period variations as well as in the retinal action current has escaped attention.

Eccles and Sherrington for the reflex centre. In their work the time course of the central excitatory state left by a subliminal stimulus proved to be a very complex process with maxima and minima at unexpected intervals between the two shocks. This, they inferred, was at least in part due to the fact that electric stimulation is inadequate in that it samples a number of fibres of mixed inhibitory and excitatory character. In this respect the eye would probably be a more satisfactory type of centre, provided the excitatory remainder can be proved to have a synaptic component.

Time and intensity, as stated above, are interchangeable for short flashes, at the threshold. It should therefore be possible by varying either factor to determine the quantity ($q = it$) of light necessary for the second flash in order that it may raise the first above the threshold. Since it is technically far more convenient to vary the duration of the second flash than its intensity, this was the procedure adopted in most of our experiments. The work was however concluded by running six control series in which the intensity of flash 2 was varied by means of thin gelatine Wratten filters covering the opening through which passed the light of the second flash. In all the last experiments the procedure consisted in 1, making flash 1 subliminal by putting in neutral tint Wratten filters with a final adjustment made by shifting the position of the lamp; 2, running flashes 1 and 2 with various dark intervals and for each interval finding the angular opening of 2 or the filter necessary for raising the after-effect of 1 above the threshold. Two important precautions in such experiments are to restrict the fixation time to a certain number of successive trials and to have the flashes controlled by the experimenter and signalled by the observer.

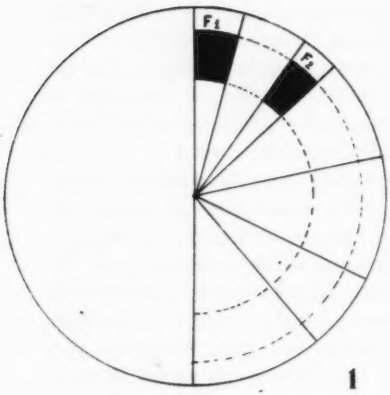
The apparatus employed was as follows: In a heavy circular metal disc a 180° slot was cut out as shown in figure 1. Concentric light metal sectors of different widths were used for varying the size of the openings 1 and 2 as well as the dark interval between them. To the axis of the disc was geared another disc—not shown in figure 1—with an opening adjusted so as to expose 1 and 2 in their proper sequence approximately every 1.8 second, when the heavy disc rotated at a speed of 6.16 RPS. The speed of the motor was controlled with the aid of a Weston electric tachometer connected to a voltmeter which gave a needle excursion of about 1½ divisions for 1 per cent alteration in speed, whereas the experimental values were determinable to only about 10 per cent. By means of a sliding rheostat, excursions of the needle were continuously compensated for. In most experiments flash 1 was kept constant at 25° of angular opening, corresponding to 11.3σ and flash 2 was varied up to the same limit, which is the range within which the relation $it = c$ holds. In a few series the motor was run at half its ordinary speed in order to obtain longer intervals between the flashes. This was compensated for by making flash 1 correspond to 12½° so as always to have the first flash lasting 11.3σ.

RESULTS. The dark adapted periphery gives the most satisfactory conditions with regard to stability of threshold which is naturally a chief

difficulty and necessitates repeated controls with flash 1 alone. No stability is obtained in less than about an hour's dark adaptation.

In figure 2 *B*, *C*, *D* and *E* relate the interval in σ between the flashes, plotted as abscissae and the duration of the second flash in σ plotted as ordinates. *B* and *C* were obtained with an area of 1°, *D* and *E* with an area of $2\frac{1}{2}°$ of visual angle. Each of these curves is the average of several experiments on the same observer. The differences between *B* and *C* and between *D* and *E* will be discussed in the next paragraph. In curve *A* the ordinates represent the duration of the second flash given as per cent of the duration of the first, the values for the curve being obtained by averaging the data of the other four curves. In the case of these four the ordinates are plotted from above downwards inasmuch as they represent the actual readings. Hence at the ordinate representing 11.3σ the stimulus is the one necessary for the threshold and the difference between the zero ordinate and any point on the curves shows how much excitation had to be added in order to reach the threshold. The difference between any point on the curve and the 11.3σ ordinate indicates the relative level of the excitatory state left by the first flash. The simple assumption has been made that flash 2 adds onto the remainder of flash 1. The value $11.3 - y$ is therefore defined as the level of excitatory remainder, y being the experimentally determined duration of flash 2. Another fairly simple definition of the level of the excitatory remainder would be to express it as an inverse function of the amount of light in the second flash, but such a procedure would only tend to smooth out significant differences in the actual readings without adding to the ease of interpreting the data.

The actual level of the excitatory state varies from individual to individual and from experiment to experiment, extremes being shown by comparing *B* with *C* and *D* with *E*. This is chiefly because of the difficulty involved in knowing precisely how far below the threshold the first subliminal happens to be. But there are also variations from day to day which cannot be accounted for and which appeared in spite of the fact that the adaptation to the dark room was started from a constant level, obtained from light-adapting to a bright screen during 5 minutes. However the time course of the process appears always to be in a general way similar, as is well shown by the average curve *A*. It first drops rapidly to a fairly steady level which with different observers runs out to between $25–40\sigma$. Then follows a slower fall and at about 130σ it is necessary to have the second flash of nearly the same strength as the first. This duration of flash 2 obviously sets a limit to the experiment. When the interval between the two subliminals was increased to $130–140\sigma$. which were the longest intervals that could be obtained with our apparatus, it was sometimes impossible to obtain summation to threshold value. However, at these long intervals the probability of eye movements interfering must be



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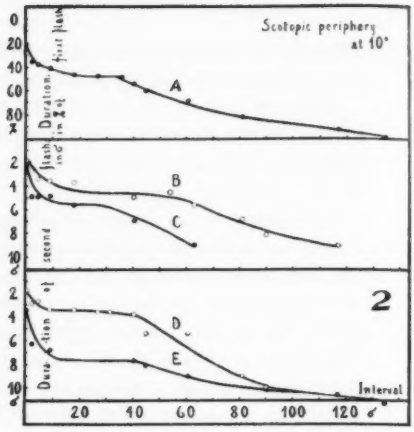

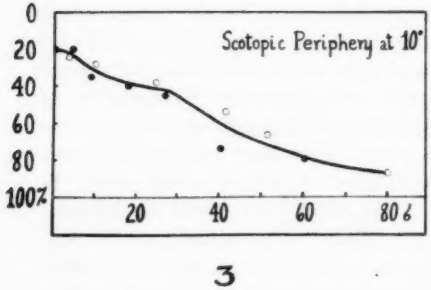

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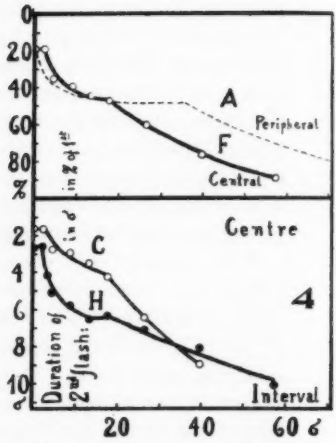

Fig. 1. Shows the rotating disc with its semicircular slot covered by a number of opaque sectors. F_1 and F_2 (black) are the adjustable openings through which passes the lightbeam. The disc rotates counter-clockwise. The size and location of F_1 is kept constant, F_2 is varied as described in the text.

Fig. 2. Experiments with observer I in the dark-adapted periphery at 10°. The subliminal flash is at about 6.6×10^{-5} millilambert. For description, see text.

Fig. 3. Experiments with observer II in the dark-adapted periphery at 10°. Black dots refer to a variation in duration of flash 2, the subliminal flash 1 being at about 1.0×10^{-4} ml. Open circles refer to a variation in intensity of flash 2, the subliminal flash 1 being at about 2.4×10^{-3} ml.

Fig. 4. Experiments with observer I in central vision, the eye adapted to about 0.03 ml. *C* and *H* are obtained with an area of 3° and 1° respectively. The subliminal flash is at about 0.021 ml., the background at the same brightness diminished by a just perceptible difference. For description, see text.

considerable. It is somewhat surprising to find that Rutenburg (1914) reports summation of two subliminals at 1300σ apart, which led us to expose our flashes only every 1.8 second. Rutenburg, in trying to determine the quantity, q, at the threshold, incidentally noted that with low intensities q decreased. This was then found to be a feature of his apparatus making the stimuli appear at shorter intervals when the intensity was diminished. The effect, as stated, could be traced up to 1.3 seconds, but it may be questioned whether he kept the number of trials constant. The duration of the flashes may also influence the time course of the excitatory remainder, particularly at long intervals where eye movements must make the probability of losing or catching a flash a function of the time it lasts.

A comparison of B and C with D and E shows that within limits the area stimulated is of little if any significance and the same result was obtained with central fixation (fig. 4) and areas related as 1:9. This does not mean that the effect of area is lacking at the threshold, for it has been well known since the publication of Ricco's work (1877) that the effect of area is considerable in compensating for low intensity of stimulation. But due to the fact that the total quantity ($i \times t \times a$) always is brought to just below threshold value and to the necessity of a constant index such as the threshold the spatial effect is already accounted for in adjusting the intensity for the first flash. The fact that the area stimulated does not materially alter the general relations found does, however, suggest that lateral diffusion of photo-chemical products hardly enters as an important factor.

Figure 3 shows that similar results are, within limits, obtained in those experiments in which the intensity of flash 2 is varied instead of its durations. The dots represent a variation in duration of the flash, the circles a variation of intensity. In these latter experiments the two flashes were of equal and very short duration (0.6σ) and the intensity of the second was varied by means of filters as has already been described.

Rapid local adaptation in the periphery makes it extremely difficult to apply our rather sensitive method to peripheral vision with a bright background. In central vision on the other hand it was found difficult to work with an absolutely black background and impossible to do so if the eye was kept dark adapted. In this latter case the fixation tends to shift to a more sensitive paracentral spot as was recognized long ago by Simon (1904). However in spite of the fact that he demonstrated unconscious deviations of considerable amplitude from the middle of the fovea, a number of workers still continue to report on the behaviour of the fovea in dark adaptation without controlling such eye movements. Because of these facts a background of about 0.02 ml. was chosen for the fovea. The results are shown in figure 4, the average curve F being plotted above the

individual averages C and H and together with the corresponding peripheral curve A of figure 2. The comparison, for reasons just stated, does not refer to identical conditions of background for A and F and only serves to emphasize the general similarity of the two. It will be seen that the actual time relations of the corresponding phases are somewhat different. A characteristic feature of the foveal curves is that the level of the excitatory remainder does not begin to drop until after $2–4\sigma$. This is sometimes noticeable in the periphery as well, where a similar short plateau is given by observer II (fig. 3). As a rule however this phenomenon is characteristic of the fovea.

Our results indicate that there are at least two factors which determine the level of the excitatory remainder as is illustrated by the two components of the curves—the fall to a plateau level and the subsequent sudden drop from this plateau. It is perhaps not mere coincidence that the break in the curve takes place at a time which corresponds approximately to that which constitutes the upper limit of the interval within which $i \times t = c$ has been found to hold. It is also worthy of note that Bloch's rule does not apply for very short exposures which cover the duration of the initial short plateau.

It is now desirable to attempt a direct determination as to whether subliminal stimuli reach the synapses and whether such stimuli are discharged laterally as is known to be the case with supraliminal ones. The method used has been to employ two semicircular test patches of 1° radius (fig. 1), 10 to 30 minutes of visual angle apart. By slightly rearranging the apparatus shown in figure 1 the two flashes have been made to stimulate the two separated semicircles. The flash applied to the one area is made subliminal and constant and the experiment consists in determining whether a similar subliminal second flash to the other area is capable of raising the remainder of flash 1 above the threshold. Care was taken that no light from one semicircle could spread over onto the other.

In spite of every possible precaution, however, the experiment was found to be too difficult to be very reliable. A great deal of time was devoted to it until we finally became convinced that the two flashes actually do sum in the dark adapted periphery at 10° and that it makes little difference whether the flashes come simultaneously or are separated by a few σ. The possibility of spread owing to imperfections in the optical system of the eye is a factor which must be considered. Whether it can explain the result or not is a question for which no definite answer can be given at this time although it must be emphasized that the defects of the optical system at oblique angles of incidence as low as 10° can hardly be serious. Aubert and Foerster (1865) performed an interesting experiment many years ago which sheds light on this problem. They projected an image of two light spots onto the retina of the excised eye of an albino

rabbit so that the images could be examined microscopically through the sclera. Thus they found that in moving the eyeball around its axis the two points appeared sharp and separated all over the retina, an observation which has recently been confirmed by Gross (1931). Aubert and Foerster concluded that the impairment of form discrimination in peripheral vision is chiefly due to properties of the retina. In a later paper in this series (Graham and Granit, 1931) it will be shown that in so far as purely physiological factors are concerned summation and its anatomical equivalent, the convergence of several frontal and lateral paths upon single neurones, offer a satisfactory explanation of the loss of visual acuity in the periphery. In our experiment it would seem as though the physiological factors are the more important because of the curious fact that the effect of separated areas upon one another could not be obtained with very long exposures even though the semicircles were separated only by a very thin black line.

As regards central vision a large number of tests were made with the faintly illuminated background of the previous experiments. The effect was however so small that it was thought statistical methods and a great number of observers would be necessary for a satisfactory treatment of the problem. We have therefore no definite results to report.

Discussion. Temporal summation seems to be as fundamental a retinal property as the effects of spatial summation discussed in the previous communications. It is obtained in different parts of the retina and with different states of adaptation. Although it is probable that part of the effect is photochemical in nature, there is little reason to assume a photochemical after-effect lasting for 130σ and taking the complicated course indicated by our curves. As shown in the last section there is also some evidence to the effect that states of excitation which are subliminal to the final conscious interpretation reach the synapses and spread laterally. This then would be an important factor in determining the well known facilitating effect of area upon the threshold. Piéron (1929) and Graham and Goldman (1931) have recently come to the same conclusion.

All the previous papers in this series show that with supraliminal stimuli synaptic factors can be traced and in some cases measured with a considerable degree of accuracy throughout the range of sensory phenomena studied. It is now interesting to see that even subliminal stimuli yield results which could be explained only with difficulty and a number of arbitrary assumptions unless the obvious conclusion is drawn that the absolute sensory threshold is ultimately conditioned by processes in the neurones, i.e., is subjected to modifications by temporal and probably also by spatial summation. The process may take place at different levels in the retina, perhaps as early as in the receptor body or at the junction between receptor and bipolar cell. Inasmuch as the effect is apparently

identical with central summation as studied by Eccles and Sherrington and by Bremer (1930) with a somewhat similar method, the absence of secondary excitatory maxima, such as found in some of their curves, is worth noting. To account for the steady state of the excitatory remainder revealed by the plateau in our curves many suggestions may be proposed. In general they will fall into two categories dependent upon whether one prefers photochemical notions to current concepts regarding the process of central excitation, either assumption being able to account for the fact that time compensates for intensity. In view of the probability of lateral synaptic spread the authors are inclined to regard the plateau as a retardation in the removal of the excitatory remainder caused by delayed impulses over lateral channels (Forbes, 1922; Eccles and Sherrington, 1931).

SUMMARY

A flash of light lasting for 0.0113 second is adjusted to a strength just below the absolute threshold of vision. When this flash is followed by a second subliminal of still shorter duration it is found that the second flash sums with the after-effect of the first to give a visible effect. The shorter the interval between the flashes, the smaller the quantity (intensity × duration) with which the second subliminal is capable of raising the after-effect of the first to threshold value. By measuring this quantity at various intervals between the flashes it has been possible to determine the level of the subliminal excitatory remainder as a function of time.

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COMPARATIVE STUDIES ON THE PERIPHERAL AND CENTRAL RETINA

V. The Sensation-Time Curve and the Time Course of the Fusion Frequency of Intermittent Stimulation

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If two beams of light, the one intermittent at a rate above the fusion point, the other continuous, are led to the two halves of a photometric field and matched, this match should hold for all exposures of the beams down to about 4σ. The experiment is due to Piéron (1928) and implies that the Talbot-Plateau law is valid during the whole course of development of the sensation. Accordingly the time course of sensation of a fused intermittent light should follow curves of the type obtained by Broca and Sulzer (1902) in their classical work on the sensation-time curve. These authors matched a flash of variable duration with a steady light and from their results plotted curves showing the development of the brightness as a function of time of exposure. Previous and later contributions to the subject are summarized in a monograph by Bills (1920). The essential point, as far as our work is concerned, is that the brightness sensation rapidly develops to a maximum which lies between $40–150\sigma$ with intensities ranging from about 30–170 lux. The stronger the stimulus, the higher the maximum, the shorter the plateau at this level, and the faster the drop to a lower level of brightness from which a more gradual fall, the local adaptation proper, sets in.

The fusion frequency, if a true index of brightness of sensation as borne out by numerous investigations, must necessarily reproduce this time course of sensation. On the other hand, there is evidence, to be pointed out later, showing that in most cases the critical frequency as an index refers to retinal processes alone, whereas the time course of sensation as determined by the method of Broca and Sulzer may involve a participation of higher centres more essential there than in the judgment of flicker or fusion.

The information that is wanted obviously concerns 1, the fusion frequency as a function of time of exposure, and 2, necessitates a determination of this function together with the sensation-time curve for the same light under identical conditions.

METHOD. The essential features of the apparatus have been previously (Granit and Harper, 1930) described. Two symmetrical 90° sectors are rotated in a light beam from a concentrated filament Mazda lamp, run at a constant voltage from a high capacity storage battery. The additional feature is a rotating shutter driven by a Telechron motor timed to make one revolution in two seconds. A mercury switch connected to a

TABLE 1

C = central, P = peripheral vision

		EXPOSURE IN σ										
		983	874	819	683	601	437	355	246	191	164	137
Area stimulated 3° of diameter												
millilamberts												
130	C	57.6	56.3		55.7	54.4	52.1		45.7		39.4	34.2
	P	62.1	61.4		60.4		56.9		47.8		38.5	29.8
13.0	C	47.9	47.7		47.0	46.2	45.5	44.7	41.7	37.4		32.6
	P	52.8	52.3		51.7	51.1	49.6	48.8	45.8	39.6		31.2
1.30	C	36.1		36.0	35.4	35.2	34.7	33.9	32.9		30.3	27.4
	P	38.9		38.5	37.6	37.2	35.7	35.4	33.8	32.6	30.7	27.1
0.13	C	27.6			27.5		26.8		25.4		23.4	20.9
	P	29.0			28.9		28.1		25.9		23.8	20.7
Area stimulated 1° of diameter												
130	C	46.3	45.5		44.9	44.1	42.4	40.3	38.4	35.6		31.6
	P	44.9	44.9		43.6	42.2	40.7	38.5	36.4	32.4		26.8
13.0	C	41.7	40.9		40.3	39.8	39.1	38.1	36.6	34.5		29.9
	P	41.6	41.4		39.8	39.5	37.8	36.8	34.8	31.3		24.8
1.30	C	31.6	31.7		30.5	30.7	29.6	28.9	26.7	25.0	23.2	20.3
	P	30.4	30.4		29.3	29.3	28.1	27.9	26.3	24.8	23.5	20.0
0.13	C	23.3			23.3		22.5		21.2		17.5	14.9
	P	23.2			23.4		22.2		21.0		16.7	13.6

press button enables the observer to operate the shutter for single exposures. The first half of the total period of rotation gives the motor ample time to attain full speed with a light disc attached to it, the second half being used for varying the exposure. The experiments were performed binocularly in moderate light adaptation (see the previous paper) against a ground glass background which reflects about 0.007 millilambert. In

our opinion these are conditions which favour repeatibility of the results over a long experimental period. A brighter background would be better still, but then low intensities could not be studied as easily.

The experiments then consist in determining the fusion frequency at different exposures of the flickering beam. At very short exposures the task is rather difficult and a great number of observations have to be taken for every single reading. This is especially the case at high intensities and the tendency there will be to underestimate the fusion point. The reason for that will later become apparent. For all intensities, however, it is true that two flashes and a dark interval are only visible as flicker when the phases are most favourably located with regard to the total time

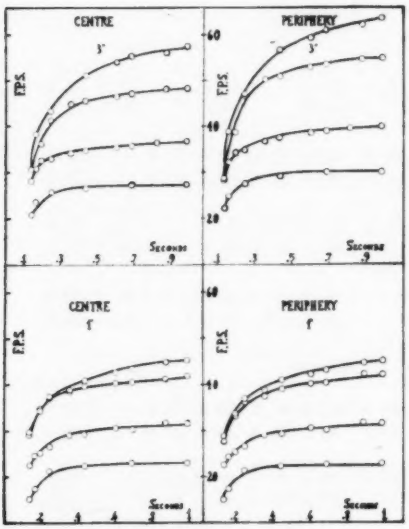

Fig. 1

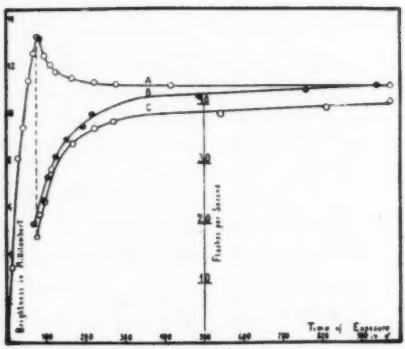

Fig. 2

of exposure. The criterion of fusion, accordingly, will have to be that in a *great number of trials* the flash must always look single. Some practice gives the required skill.

Results. Typical results are shown in figure 1, and table 1 summarizes about 1100 individual determinations averaged from the data of the two observers. In the figure the diameter of the circles indicates the mean variation except in the case of the shortest exposures at high intensities where the mean variation is greater. The curves represent data obtained in a series of experiments on one observer and are for a 3° and 1° area in the center and with peripheral fixation at 10°.

The general result is clear cut. The fusion frequency increases with

time of exposure. The higher the intensity the longer the time necessary for reaching the maximal critical frequency even though the rate of rise is faster than at low intensities. There is also as stated above a relatively greater drop in the high intensity curves at short exposures. Increase of area stimulated affects the curve as does an increase of intensity and the moving of a *large* area towards the periphery. As previously pointed out and explained in this series (Granit and Harper, 1930) the difference between centre and periphery is a function of area and intensity of the stimulus.

With regard to our main problem it is evident that not only do these curves rise at a time when the ordinary sensation-time curves (Broca and Sulzer) fall, but, furthermore, the maximal fusion frequency is reached later at high than at low intensities. This also is precisely a reversal of the findings with the equality of brightness method and continuous light as used by Broca and Sulzer.

Comparison of flicker and sensation-time curves. In view of the results obtained in the experiments just described an apparatus was designed to make possible a determination of the sensation-time curves as well as of the time course of the critical frequency. Since this apparatus is a convenient unit for a number of different experiments on flicker and retinal interaction, all of which can be easily repeated and demonstrated, it is being described in a subsequent paper. At present it is sufficient to say that the light beam was split in two by means of prisms, with Telechron motors introduced into the one beam together with their respective shutters and timed to give different speeds in order to obtain the total range of exposures wanted.

By utilizing lenses the shutters were made to cut the beam at the focal point where its cross-section was only a few millimeters. The other beam was exposed throughout an experiment as comparison field and its intensity was adjusted by means of a carefully calibrated wedge covering a range of 1:2. A small balance wedge ensured homogeneity of the utilized section of the beam. Coarse adjustment was obtained with filters. A flashed opal glass was substituted for the ground glass on account of the fact that judgment as to brightness-differences is facilitated the more the surface approaches that of a perfect diffuser. Either or both beams could be flickered by introducing the rotating sectors at the desired point in the system.

To the observers the lights appeared as two adjacent semicircles together subtending 2° of diameter and seen against a fairly bright background of 0.195 millilambert. The experiment consisted in making a brightness match between the steady half and the flash at various times of exposure of the latter. This having been done the flash was made to flicker and its fusion point determined for various exposures. Central fixation and monocular vision were used throughout these experiments. Conditions in general were chosen so as to make for greatest possible accuracy. The

flickering semicircle was matched approximately with the steady half at each exposure so as to make the conditions with regard to interaction as comparable as possible in the two sets of experiments. It was found furthermore to be a great advantage to have a steady comparison field by which to judge as to flicker or fusion at short exposures. It was now possible to determine with a fair degree of accuracy the shortest exposure for flicker to appear.

The sensation time curve as averaged from 120 readings with two observers is plotted as A in figure 2. The brightness at the steady level[1] is 11.1 ml. This light was then flickered and the fusion point determined as a function of the time of exposure. Curve C gives the result. A shows how

TABLE 2

EXPOSURE	RISE OF BRIGHTNESS	EXPOSURE	INTERMITTENT LIGHT AT 11.1 ML.	EXPOSURE	INTERMITTENT LIGHT AT 22.2 ML.
σ	*millilamberts*	σ	*flashes per second*	σ	*flashes per second*
976	11.1	976	40.3	920	43.0
417	11.1	813	39.6	760	42.3
278	11.2	542	38.4	487	41.3
222	11.3	271	37.0	217	38.2
167	11.5	222	36.0	194	36.3
125	11.7	167	33.4	153	34.1
111	12.0	111	29.0	125	31.1
97.3	12.4	97.3	23.7	104	27.7
83.4	13.2	83.4	21.3	90.4	23.9
76.5	13.2	76.5	17.7	69.4	19.9
69.4	12.5				
55.6	11.4				
41.7	9.4				
27.8	8.0				
13.9	3.4				
0	Background at 0.195				

the first flash of C would rise if not restricted by the criterion of fusion. But C, of course, would always appear darker than A, since, on the Talbot Plateau law, the two 90° sectors reduce its brightness by 50 per cent. Hence, in order to obtain the flicker-time curve of a light which is of equal brightness with A, a light of 22.2 ml. was flickered. The result is given by curve B. The two flicker curves are averages of 160 observations. The data of figure 2 are tabulated in table 2.

[1] Bills (1920) prefers to plot the rise of the sensation in terms of just perceptible differences. The authors see no particular advantage in replacing brightness as measured photometrically by a still less interpretable unit. Bills also objects to the concept of the "steady level." However, both this method and the flicker method (Granit and v. Ammon, 1930) show that in *central vision* there is no drop in brightness between 1–3 seconds.

The curves show, as did the matching of the steady patch with the flickered half at the fusion point, that the two functions are altogether different. If A and C were equalized with the comparison field at about 1 second then it was necessary in both cases to raise the brightness in the comparison field at short exposures, just as shown by curve A, and this in spite of the fact that the fusion frequency as given by curve B or C was rapidly decreasing through that same interval. The brightness of the fused sensation obtained with intermittent light thus followed, at least in a general way, the sensation-time curve of the continuous stimulus. In other words, the time course of brightness in curve B follows approximately the course of brightness sensation shown in curve A. In order to find out precisely how A was matched by B, Piéron's experiment was repeated but with the flickered patch kept just *at the fusion point*. It was first matched with the steady comparison field at infinite exposure and the exposure then cut down for both simultaneously. At each stage the two were compared. Within the limits of error they were found to be approximately equal, except at the short exposures corresponding to the lower branch of the flicker curve where the fused intermittent light looked decidedly brighter. But as soon as the sectors were rotated at very high speeds, far above the fusion point, the brightness match established at long exposures again was fairly satisfactory at short exposures also.

The significance of this observation will become clear when the actual time relations are set forth. Let us consider the lowest point on B which is at a total exposure of 69.4σ and corresponds to a fusion frequency of 19.9 flashes per second with 50.3σ for each flash as well as for each dark interval. Even the most unfavourable sequence of phases, 9.5σ light, 50.3σ dark, and 9.5σ light, totaling 69.4σ, is perceived as a fused sensation, brighter than the one given by a 50 per cent less intense light, A, exposed continuously for 69.4σ. The total exposure of the flickered light can vary between 19.0 and 50.3σ with variation in the relative phases. If the quantity of light rather than the intensity were decisive these figures should be multiplied by 2 because A is at 50 per cent of the intensity of B and the range of variation would then be from 38.0 to 100.6 units. In this case only a certain percentage of the observations could give a greater brightness for the flickered patch, the steady patch being constantly illuminated for 69.4σ corresponding to 69.4 units in terms of quantities ($i \times t$). Obviously then it is not sufficient to multiply by 2 and the total quantity of light therefore does not determine the brightness at short exposures, but rather the rate at which this quantity enters the eye.

This conclusion is well substantiated by evaluating the curves published by Broca and Sulzer. They obtained sensation-time curves over a range of intensities and plotted them as above. Now, if the product of intensity and time of exposure were a constant ($it = c$), then a line parallel with the

abscissa and cutting a family of sensation-time curves obtained at different intensities of stimulation gives a constant level of brightness which is the index wanted for the product $i \times t$. In this way it is possible to determine whether a constant level of brightness corresponds to a constant amount of energy or whether the rate of the processes concerned is also significant. If the ordinate used as index be about 12 lux it will be found by graphical examination of Broca and Sulzer's curves that for the intensities 170,126 and 64.5 lux the products $i \times t$ relate roughly as 17:29:82 for the constant level of brightness. Mere inspection of their curves shows that the relation is of this type. The higher the intensity of stimulation, the greater the rate at which the processes underlying brightness develop.

Such a relation is evidently to be expected from work on other sense organs. Direct evidence has been obtained by Adrian (1928) and by Matthews (1931) by recording nerve impulses from muscle spindles and pressure end organs. On the sensory side there are also the results obtained by v. Frey on touch (1929). Furthermore, as is well known, the rate of stimulation determines the efficacy of a muscle or nerve stimulus.

We may safely conclude that at short exposures the rate of stimulation is relatively more important than the total amount of energy and that the sensation-time curve expresses this fact. How precisely, we do not know, since it is not clear whether at each exposure the brightness match refers to the final level of brightness reached at the end of that exposure or is some complex integrated function of all the stages the process has traversed during the total time of exposure. At long exposures the rate of stimulation, of course, loses in significance.

Whereas the general nature of the sensation-time curve is indicated in a fairly direct way by the experimental evidence it is more difficult to know what lies behind the fusion frequency-time curves. The time relations of the "peak" of the sensation-time curve show that the fusion frequency is not determined by the fusion of such peaks since the frequency at this point would be only about 11 f.p.s., whereas the corresponding critical frequency actually is about 23 f.p.s. for curve *C*. On the other hand the rate of rise of the sensation certainly is reflected in the fusion frequency. Thus Ives (1922) found that when rotating sectors were cut so as to give the same mean brightness but brought the light on more or less gradually, the one which produced the most rapid development of illumination gave the highest critical frequency. The effect, however, also depended upon the amplitude of the wave, the rate of onset being relatively more important at small amplitudes. In the retinal action potential Piper (1911) found the "peaks" in flicker to correspond to the rapidly rising b-waves in the well known onset of potential change. The criterion of fusion implies, as shown by our results, that the rising phase of the sensation-time curve is cut off before the maximum has been reached. The only direct correla-

tion between the peak in *A* and curve *C* is that the shortest time within which flicker can be perceived is identical with the time necessary for the sensation to reach its maximal point (cf. the vertical line in fig. 2). The peak, of course, will seriously interfere with the perception of flicker at high intensities and short exposures. Thus the flicker curves at high intensities will become relatively too low at short exposures (cf. fig. 1).

Two facts which on the surface appear to be contradictory will have to be kept in mind when discussing the nature of the fusion frequency as an index of brightness. The one is that an array of experimental evidence, part of which was mentioned in a previous paper in this series (Granit and Harper, 1930) indicates that the fusion frequency records the intensity of the physiological process underlying the perception of brightness. The other one is the very striking fact, illustrated by figure 2, that at certain exposures the fusion frequency and the brightness perceived alter in a different direction (cf. curves *A* and *C* in the region following the peak). The latter observation may be amplified by the following experiment: If at an exposure of about 1 second a brightness be chosen which gives a fusion frequency of about 20 f.p.s. it is found that, when this critical frequency is located on curve B, the sensation as given approximately by *A* corresponds to about 13 ml. The brightness which has to be used at the long exposure for giving the same response in terms of fusion frequencies is then only about 0.3 ml. How is it possible that the fusion frequency can be a criterion of brightness and at the same time not correlate in a given way with brightness? Undoubtedly equal fusion frequencies must imply that the processes responsible for fusion are equal. The individual peaks in the flickered lights must for a given fusion point rise from the same initial level to the same height and they must leave excitatory remainders of equal duration to account for the fact that equally long dark intervals are non-visible. The time course of such excitatory after-effects was plotted in the previous communication (Granit and Davis, 1931) for subliminal flashes.

The answer to all these questions can only be given by an assumption fairly well limited by the above considerations, namely, that the fusion frequency is an index showing the level of an excitatory state in the retina. There seems to be no way of relating fusion frequency to brightness unless it be assumed that this is the case. Owing to the fact that the sensation at short exposures is determined by the rate at which the excitatory state is formed, and to the participation of higher centres into which this "reaction to the rate" is propagated, the brightness at short exposures does not correlate with the level of excitatory state in the retina.

As a matter of fact all the evidence available shows that under simple conditions the fusion frequency is determined by retinal processes alone. Sachs (1929) states in a short communication (based on work which is

extensively reviewed by Kohlrausch, 1931) that in taking action potentials from his own eye, as previously done by Hartline (1925), flicker as perceived corresponded to small waves superimposed upon the action potential whereas fused intermittent light gave the ordinary smooth wave. Kohlrausch concludes that the flicker method reproduces purely retinal conditions. Adrian and Matthews (1928) found that the fusion point defined as asynchronism of impulses arriving through the optic nerve of the eel's eye behaved, with regard to area and intensity, like the seen fusion point of the visual experiment. Piper (1911) has published extensive series of retinal action potentials obtained from various vertebrate eyes in response to intermittent stimulation. Here as in the work of Sachs flicker is found to be small waves superimposed upon the slow phase of the action potential and at certain rates of intermittency fusion appears. In addition there is the fact, noticeable, for instance, in his figures 20–22, 25, 27–29, 36, 37, that a constant rate of flicker does not burst through the action potential until several flashes have passed. This, of course, is precisely what on the basis of our experiments should be expected.

It would seem, therefore, as if our experiments should be repeated on excised eyes before the theoretical analysis is carried further. If then new correlations could be established our results might serve as a basis for quantitative treatment. Purely empirical equations, in our opinion, deserve little attention. As to the general question whether the process is localized in the retinal neurones or in the receptors, the former assumption seems more probable, since it is easy to show by taking two areas some distance apart that the synaptic lateral components influence these flicker curves considerably (cf. also previous communications by Granit, 1930; Granit and Harper, 1930; and the following paper in this series, Graham and Granit, 1931).

The authors are indebted to Dr. D. W. Bronk for valuable suggestions and to Mr. A. J. Rawson for technical assistance and perfection of apparatus.

SUMMARY

The development of the fusion frequency as a function of time of exposure has been studied in central and peripheral vision with a 1° and a 3° area.

The fusion frequency increases first rapidly, then slowly with time of exposure. The higher the intensity, the steeper the rise and the longer the time to the maximum.

Increase in area affects the time course of the fusion frequency as does an increase in intensity and as does the moving of a large area towards the periphery (at 10°).

The curve showing the development of the sensation of brightness has

been obtained under conditions identical with those used in measuring the time course of the critical frequency. In plotting the two sets of data to the same abscissae (time of exposure) and correlating them it becomes evident that the two functions are different, in spite of the fact that the flicker phenomenon is an index of brightness. The nature of this discrepancy is analyzed.

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COMPARATIVE STUDIES ON THE PERIPHERAL AND CENTRAL RETINA

VI. Inhibition, Summation, and Synchronization of Impulses in the Retina

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From the Eldridge Reeves Johnson Foundation for Medical Physics, University of Pennsylvania

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It has been pointed out in a previous communication of this series (Granit and Hammond, 1931) that there is a sufficient body of evidence to warrant our taking the standpoint that, under simple conditions, the fusion frequency of an intermittent light is determined by purely retinal factors. There is therefore no reason for believing that the higher centres should split up and synchronize a discharge which arrives through the optic nerve as an asynchronous continuous discharge (Adrian and Matthews, 1928) except in the case where the two eyes are stimulated by lights flickering in varying phase, as in the classical work of Sherrington (1904–5) on binocular flicker. But even in this case it appears that the fusion frequency was altered little in any except a few well specified cases. Similarly, Allen's (1919) method of studying the effect of "fatigue" on flicker may introduce complications.[1] It therefore seems necessary to begin with to select conditions which give reasonable assurance that only retinal factors are involved.

Previously in this series (Granit, 1930; Granit and Harper, 1930) it was shown that two or more separated flickering areas interacted so as to give a higher fusion frequency for all of them stimulated simultaneously as compared with the value obtained with each area alone. Here we are raising

[1] With his procedure of "fatiguing" the flickering area, adjoining areas and even the other eye, Allen has obtained a number of interesting and curious results which we hesitate to discuss because of our lack of experience with the conditions under which the experiments were made. Allen takes, for example, the normal fusion frequency curve for the spectrum as his reference standard. Then the eye is "fatigued' for one wave-length, and in testing again for the fusion frequency throughout the spectrum, he finds that it is lowered for certain colors. This amounts to finding *selective fatigue* for these colors. The difficulty met with in interpreting such results is that selective fatigue has not been found in the excised eye. Fatiguing with one color affects equally the action currents for all colors (Chaffee and Hampson, 1924). As to Allen's explanation, see the discussion.

the question as to whether the fusion frequency of a given area can be increased also by illuminating an adjacent area with steady light. Further, considering that histologically the retina is a nervous centre it would seem reasonable to expect inhibitory processes in it. This, with the flicker method would amount to a search for conditions where one area would influence another so as to lower the fusion frequency in the latter. The experiments to be summarized demonstrate this latter activity as occurring under conditions which are slightly different from those productive of summation.

METHOD. Figure 1 shows the arrangement of apparatus. The light from the Pointolite lamp, *L*, is divided by four 45° totally reflecting prisms into two beams which are centered on semicircular apertures in the opaque screen, *A* (cf. arrangement in fig. 2). Adjustment of the outer prisms allows for the focussing of the rays upon the semicircles at any separation. A series of screens *A* (shown separately in fig. 2) with opaque graded distances between the semicircles provides opportunity for determining inter-

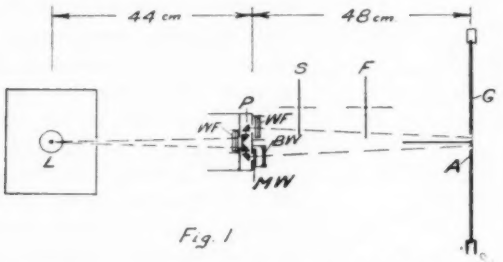

Fig. 1

action effects with different separation. *G* is a plate of flashed opal or frosted glass. Associated with the prisms are two holders for standard Wratten filters *WF* and a Wratten wedge filter, *MW*. The latter is adjustable by a rack and pinion. *BW* is a small balance wedge. A shutter and the flicker apparatus which may be placed in any section of the beams are indicated in the figure by *S* and *F* respectively. The shutter has been in the unsplit beam in the experiments reported below, and used only in those described in table 1. Long exposure was found to enhance the effects to be described.

Figure 2 shows the removable section of the opaque screen *A* with the semicircular openings. Their radius corresponds to 1° of visual angle. The fins *FF* prevent reflection from *D* at glancing incidence. In the same figure appears the mechanism of the shutter. *SS* is the shutter shaft driven through reduction gears by a Telechron synchronous motor in the direction shown until stopped by the pin, *SP*. At this point the mercury switch *MS* is opened by the cam *C*. When the button *B* is closed by the observer, the relay *R* operates. This releases the pin *SP* and closes the contacts *CP*, *CP*, thus shunting the mercury switch and starting the motor. As soon

as the shaft has turned slightly the cam C allows the switch MS to close and the motor continues to run until the shaft SS has made one complete revolution.

The technical perfection of the apparatus described is due to Mr. A. J. Rawson to whom the authors wish to express their thanks.

Devices for measuring and controlling the flicker apparatus and the general conditions of the experiments have been described previously (Granit and Harper, 1930). The lamp was run on a high capacity storage battery. Monocular vision was employed throughout the work. High intensities have been used because only under these conditions is it possible to deal with effects which are unquestionably beyond the range of experimental variations.

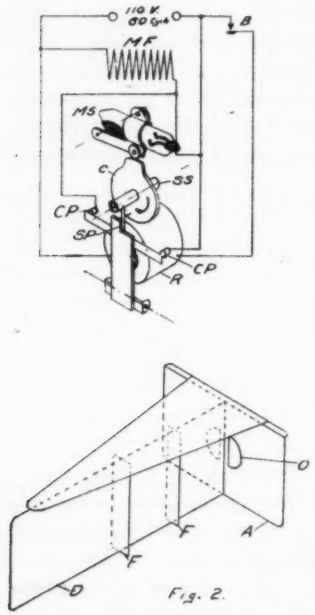

Fig. 2.

Synchronization and summation. In table 1, as in all the following tables, the fusion frequency is given in flashes per second as an average of results obtained with a number of observers (3–4). Two flickering semi-circles of equal intensity (rotating sectors in unsplit beam) give, if taken together, an increase in fusion frequency of 5.9 per cent over either singly, when regarded in the fovea, and 13.7 per cent in the periphery at 10°. Comparing these values with the results obtained when one semi-circle alone is flickering (rotating sectors in split beam), and the other one steadily illuminated and matched with the flickered patch as fused, it is evident that the foveal interaction has been reduced to 2.9 per cent and the peripheral to 6 per cent.

We may thus conclude that interaction via lateral paths is greatly favored by intermittent stimulation. Part of the interaction may therefore be a matter of synchronization of impulses within the individual areas stimulated (Granit and Harper, 1930). There is, however, no method for deciding with sensations as criteria, whether this synchronization involves summation or not since direct comparison, a relative method, only gives information as to the differences in the two areas compared. But the fact that a continuous discharge of impulses in one area is also capable of raising the fusion frequency in an adjacent flickering area shows that synchronization of impulses in the adjacent area is not essential for the effect. At least in this latter case, and probably in the former also, the

interaction must be a true spatial summation which implies that a higher state of excitation may be produced in a given area through the agency of impulses from any area reasonably near to it (cf. the results by Lythgoe and Tansley, mentioned in the discussion).

Inhibition. The fact that two flickering semicircles some minutes apart on the retina may give practically maximal summation and yet appear

TABLE 1

Conditions: Both semicircles at 35.0 millilamberts, background at 0.012 ml. Semicircles about 4 minutes of visual angle apart. In this and in all the following tables the percentage increase or decrease is the difference between the "doubles" and the "singles" in per cent of the "singles."

	ONE SEMICIRCLE FLICKERING, THE OTHER STEADY				BOTH FLICKERING			
	Centre		Periphery		Centre		Periphery	
	Single	Double	Single	Double	Single	Double	Single	Double
Fusion frequency	49.1	50.5	46.7	49.5	49.5	52.4	46.7	53.1
Percent difference	2.9		6.0		5.9		13.7	

TABLE 2

Conditions: Left semicircle always at 139 ml., background at 0.012 ml. Both flickering and about 4 minutes of visual angle apart. Central fixation.

SINGLES	DOUBLES	PERCENT DIFFERENCE
Both equally bright		
55.8	60.6	+8.6
Right 50 per cent darker than left. Fusion frequency of right determined		
54.7	53.9	−1.5
Right 50 per cent darker than left. Fusion frequency of left determined		
55.8	58.8	+5.4

distinctly separated has been noted in a previous paper (Granit and Harper, 1930). It was also pointed out that it is difficult to understand how visual acuity can increase with the intensity of the stimulus when the summative process also becomes more marked at these higher intensities. The same difficulty holds for brightness discrimination. These facts suggest that, as soon as the excitatory processes in adjacent retinal areas are at different levels, conditions may be present which differ fundamen-

tally from those productive of maximal summation. Fortunately the experimental test is easy to make.

Two flickering semicircles (sectors in unsplit beam) are kept at different levels of brightness. The darker semicircle will give a lower fusion frequency owing to its lower intensity. But the difference between the two can be made negligible by working the whole experiment at a high intensity where the fusion frequency is practically independent of intensity (Granit and Harper, 1930). Thus in table 2 the one semicircle alone gives 55.8 f.p.s. When the illumination on it is diminished by 50 per cent, the reduction in fusion frequency only brings it down to 54.7 f.p.s. If both then are equally bright, as is the case in the upper part of the table, here

TABLE 3

Conditions: Right semicircle flickering and at a brightness of 13.9 ml. (upper part of table) which in the lower part of the table is reduced by 33.3 per cent, whereas left is kept unaltered throughout the experiment and matched to give the brightness of the right patch fused at 13.9 ml. The semicircles are about 4 minutes of visual angle apart. Background at 0.012 ml. Central fixation.

RIGHT ALONE	RIGHT WITH LEFT ILLUMINATED	PERCENT DIFFERENCE
Both equally bright, when right fused		
47.8	50.0	+4.6
Right reduced by 33.3 per cent in intensity. Left as above		
45.6	42.2	−3.1

is a summation on either of them amounting to 8.6 per cent. But if the one semicircle is at a 50 per cent lower intensity its fusion frequency does not increase in the least when the brighter half is simultaneously illuminated. As a matter of fact it has diminished by 1.5 per cent[2] as shown by the second line of the table. This becomes particularly striking by comparison with the behaviour of the fusion frequency read on the brighter semicircle in the same experiment, shown in the lower third of the table. When the darker semicircle is illuminated it raises the fusion frequency of the brighter by 5.4 per cent. The result is clear cut and shows that when two adjacent areas are at different levels of brightness the interaction is of such a nature as to exaggerate the difference between them.

[2] The diminution owes its appearance in the averaged values to one of the observers (E. H.) who constantly gave it with intensity differences between 25–50 per cent. Two of the observers repeated with the doubles and the darker determined the value obtained with the darker single. The fourth observer (R. G.) often had a just measurable decrease with the darker in the double constellation.

It is interesting to note that the result essentially corresponds to the picture of contrast and that we probably have here a retinal component of this complicated mechanism. It seems necessary to classify the phenomenon as inhibition since the total effect of the interaction on the darker is removed and since part of it at least, as has been shown, is excitation. Two "darker" semicircles if *equally* "dark," in this experiment would give a summation somewhere between 5.4 and 8.5 per cent. This, as it were, is the background against which the inhibition has to be pitted in order to become recognized.

This general type of experiment was repeated many times under somewhat different conditions. Inasmuch as the experimental variations were found to shed light on some aspects of the problem they are reported below.

TABLE 4

Conditions: Right semicircle in upper part of table at 139 ml. reduced by 25 per cent and by 33.3 per cent respectively in middle and lower part of table. Left steadily illuminated and unaltered throughout the experiment as matched to give the brightness of the right patch fused at 139 ml. Semicircles about 8 min. of visual angle apart. Background at 0.012 ml. Peripheral fixation.

RIGHT ALONE	RIGHT WITH LEFT ILLUMINATED	PERCENT DIFFERENCE
Both equally bright, when right fused		
52.4	55.4	5.7
Right reduced by 25 per cent. Left as above		
51.5	54.4	5.6
Right reduced by 33.3 per cent. Left as above		
50.8	53.6	5.5

Table 3 gives data obtained with the one semicircle steady and the other one flickering, central fixation again being er ployed. The summation with both equal was 4.6 per cent. Then the intensity of the flickering patch was reduced by 33.3 per cent. Alone it now gave a fusion frequency of 45.6 f.p.s. as against 47.8 before the reduction. When the brighter semicircle was illuminated as in the upper half of the table its fusion frequency fell to 44.2 f.p.s. The fall below the value obtained with the single is mainly due to one observer. With the other ones the inhibition only removed the summated surplus.

In the periphery this type of experiment is difficult to perform if both patches are flickering, since it presupposes a degree of discrimination between flicker and non-flicker in adjacent areas which the periphery does

not possess. But it can be performed as above, with one area steady and the other flickering, although preferably with the patches somewhat farther apart. Such an experiment is shown in table 4. Independent of whether the patches are equal or the flickering one is reduced in brightness by 25–33.3 per cent they sum and the summation is fairly constant, 5.5–5.7 per cent. Similar results are always obtained in the periphery, even with larger differences in brightness.[3] Inhibition therefore appears to be chiefly a foveal property, at least at higher intensities. In accordance with this observation is the fact that visual acuity and brightness discrimination

TABLE 5

Conditions: Both flickering and at 23.3 ml. in the upper part of the table. In the lower part the right is reduced in brightness by 2 per cent. The semicircles are separated by a very thin line. The background is at 0.195 ml. Central fixation.

Both equally bright

SINGLES	DOUBLES	PERCENT DIFFERENCE
44.9	47.5	5.8

Right reduced in intensity by 2 per cent, left unaltered

RIGHT ALONE	RIGHT WITH LEFT ILLUMINATED	PERCENT DIFFERENCE
45.0	46.0	2.2

Right reduced in intensity by 2 per cent, left unaltered

LEFT ALONE	LEFT WITH RIGHT ILLUMINATED	PERCENT DIFFERENCE
45.0	47.4	5.3

are also special functions of the fovea and very much less developed in the peripheral eye.

For brightness discrimination much smaller differences are required than those hitherto studied. The just perceptible difference with a thin line of separation between the patches as in our apparatus amounts to a diminution of the intensity in the one patch of about 2 per cent for medium and high intensities. Table 5 shows how the fusion frequency of one semicircle is altered by the presence of *1* an equally bright adjacent semicircle (upper line), and *2*, of a just perceptibly brighter semicircle (second line).

[3] With diffusing surfaces such as ground or flashed opal glass it is impossible to work with very great differences in brightness between adjacent areas uuless the line of separation cuts through the glass. In the centre levels of brightness differing as much as 1:100 were tried and the result was very much the same as when the patches differed only by a relation of 1:2.

With both equally bright there is a summation of 5.8 per cent. As soon as the right semicircle is made just perceptibly darker than the left, the fusion frequency read on the darker shows the summation reduced to 2.2 per cent, whereas if the readings are taken with the brighter there is still a summation amounting to 5.3 per cent.

Discussion. The interesting fact that synchronous stimulation of adjacent areas favours lateral interaction between retinal neurones is at present a rather isolated observation in the field of central nervous physiology. There is, however, the finding of Adrian and Matthews (1928) that synchronization of discharge may develop spontaneously in the eel's retina if large parts of it be stimulated. On the motor side Adrian and Bronk (1928) found the respiratory centre to discharge rhythmically, if strongly stimulated by asphyxia. Above it was suggested also that, vice versa, the establishment of synchronization, as by flicker, increases the excitatory activities of a centre and that, hence, the facilitated interaction means increased summation. The relations between our results and those mentioned above may be incidental, but taken together they all stress the importance of a factor in reflex work which hitherto has received but little attention.

The presence of inhibitory processes in the retina brings in one more connecting link between the physiology of sensory and motor neurones. It seems necessary to conclude that there is in the retina an inhibitory pathway and furthermore, that this pathway is especially well developed in the fovea. There is, indeed, in this region a system of cells, the amacrines, which here is more developed than elsewhere in the retina (Greeff, 1900), and which is joined to the efferent fibres in opticus. These fibres as well as the amacrines must have some special function. Whether the inhibitory effects have descended from higher central stations or not is of no immediate concern, but our results certainly give a great deal of justification for localizing the inhibitory functions in the amacrines. Allen (1923) holds that the efferent fibres carry both excitatory and inhibitory effects.

The fact that inhibition requires different levels of excitation in adjacent neurones in order to appear would seem to have the practical significance that visual acuity and brightness discrimination are possible in spite of summation over lateral channels. It is another indication of the extreme complexity of the processes underlying these visual functions. The theoretical significance of the observation seems to be that the inhibitory system is excited relatively more and more as the intensity of the excitatory process in a group of neurones increases. Consequently the inhibitory effect passing from the more intensely stimulated area to the adjacent less stimulated area will be greater than the inhibitory effect passing in the opposite direction. Thus only is it possible to picture in general terms the conditions which make inhibition measurable.

Our results may be said to describe a mechanism of contrast in the retina, but it should be borne in mind that such phenomena as binocular contrast and contrast in the blind spot show that there are essential cerebral factors in contrast which can hardly be measured by the flicker method. Sherrington's (1897) ingenious experiments with the flickering "contrast discs" in our opinion do not show once for all that contrast-effects can be measured by this method. They may be explained by the fact that phase-differences were introduced between the adjacent flickering areas or they may be effects of the type described above. That contrast effects as a rule cannot be measured by the flicker method is shown by the work of Lythgoe and Tansley (1929), who found the fusion frequency to *decrease* when the illumination on the background was diminished. By contrast the test patch at the same time must have appeared brighter. The summation, however must have diminished, as shown above, and this factor may account for the results they obtained.

Finally it should be noted that on the strength of the evidence in this paper the low visual acuity and brightness discrimination in the periphery may be explained as being due chiefly to summation. In a previous paper in this series (Granit and Harper, 1930) we hesitated to take this standpoint because of the fact that summation is present in the centre also. Seeing now that the centre possesses an additional mechanism for rendering the summation inactive under conditions where visual acuity is called for this point of view appears rational. At 10° the frontal convergence of receptors is too low to account alone for the low visual acuity (cf. Chiewitz's table in the previous paper). As a matter of fact the visual acuity begins to decrease rapidly from the middle of the fovea (Wertheim, 1894) before the frontal convergence in the periphery can be made to account for it and before the increased peripheral scatter of light is of immediate significance.

SUMMARY

An apparatus has been used in which two beams of light led to two adjacent semicircular test patches can be varied independently with regard to intensity and flicker.

When the two semicircles are of equal brightness and flickered synchronously they give a higher fusion frequency than each taken singly. When only one beam is flickered and the other one adjusted to give the same brightness as the flickered patch when fused the presence of the steadily illuminated semicircle raises the fusion frequency of the flickering semicircle. In this case, however, the effect is less than in the case when both are flickered.

It is concluded that synchronization of impulses favours interaction over lateral channels. Since a steadily illuminated area is capable of rais-

ing the fusion frequency in an adjacent area, it is further concluded that part at least of the interaction, but probably the whole process, involves a true spatial summation.

When the two semicircles are at different levels of brightness the fusion frequency of the darker is unaltered or lowered by the presence of the brighter, whereas the fusion frequency of the brighter is increased by the presence of the darker, provided the difference between them is not too great. The removal of the summation on the darker must be due to inhibition, since the results obtained with the brighter show that summation is bound to appear unless actively inhibited. Also the "darker" semicircle would sum with an equally "dark" adjacent patch.

By showing that when two adjacent areas are at different levels of brightness, the brightness difference is exaggerated by a specific mechanism, which well may be a retinal form of contrast, the results explain how it is possible at high intensities to retain visual acuity and the faculty of discriminating brightness in spite of the increased summation over lateral channels.

The inhibition is especially well developed in the fovea. No evidence of inhibition at high intensities in the periphery has been obtained.

The predominance of summation in the periphery explains its low visual acuity and deficient brightness discrimination at degrees of eccentricity where convergence of receptors and scatter of light are of less significance.

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THE RELATION OF CORTIN TO THE MAINTENANCE OF BODY TEMPERATURE[1]

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It is well known that adrenalectomized mammals are unable to maintain their body temperature as well as normals. This inability becomes so great in the late stages of adrenal insufficiency, that the fall in temperature may be quite marked (Elliott).

Animals that die soon after operation show a more abrupt fall while those that live for a considerable length of time tend to have merely a subnormal temperature. This is true even in a warm room (27°–28°C.). All adrenalectomized mammals whether they live a short or long period show a very low resistance to cold. The responsibility for this faulty temperature regulation has not yet been established.

Epinephrin, the substance first obtained from the adrenal gland might be responsible, at least in part.

Cramer found histological evidence which indicated greater utilization of epinephrin in normal animals exposed to cold. Those which resisted cold poorly showed depletion of the "adrenalin granules" while those that resisted cold well did not show such depletion.

Adrenalectomy removes the principal source of epinephrin and therefore could account for the failure in heat production under these conditions.

Direct evidence of increased epinephrin secretion in response to cold was furnished by Hartman, McCordock and Loder by means of the completely denervated iris. Exposure to cold caused a marked increase in the output of epinephrin. Peripheral stimulation rather than fall in the general body temperature seemed to be responsible (Hartman and Hartman). Cannon, Querido, Britton and Bright by means of the denervated heart also demonstrated an increase in the output of epinephrin upon exposure to cold.

Injected adrenalin increases heat production (Boothby and Sandiford); moreover reflex stimulation of the adrenals, which may increase the epinephrin output, augments metabolism. It was therefore reasonable to infer

[1] Aided by a grant from the Ella Sachs Plotz Foundation.

"that absence of the medulla and its secretion, rather than absence of the cortex, is responsible for slower metabolic rate after removal of the adrenals" (Cannon, Querido, Britton and Bright).

The first direct evidence that it is the loss of cortex rather than medulla which is responsible for the lower resistance to cold after adrenalectomy was furnished by Wyman and Tum Suden. They demonstrated that adrenalectomized rats could be protected against short exposure to cold by autoplastic cortical transplants.

In the present investigation we have studied the rôle of cortin (Hartman, Brownell and Hartman) in the maintenance of body temperature. Albino rats were chosen as the test animals because they are easily obtainable in large numbers, of uniform stock and of any desired age; they occupy a small amount of space which is important particularly when moderately high or low temperatures are to be maintained artificially; and they require a small amount of cortical extract per animal.

In each group of experimental animals it is necessary to choose individuals of approximately the same size and thickness of coat so that the heat loss will be comparable. These requirements are fairly well met by using rats of the same age. Struggling must be avoided when temperatures are taken because of the attendant increased heat production.

The animals used were two to three months old, and weighed 100 to 175 grams. They were reared in a temperature of 27° to 28°C. kept fairly uniform by a thermostat. Double adrenalectomy was performed at a single operation by the dorsal route in three to five minutes. Food and water were available to them at all times except when the animals were exposed to cold or previous to the determination of metabolism.

All injections were made subcutaneously unless otherwise stated. Cortical extract contained the product of 50 grams of cortex per cubic centimeter. Injections were usually made twice daily, viz., at 9 a.m. and 9 p.m.

A chemical thermometer 17.5 cm. long, graduated in degrees but estimable to tenths, proved more satisfactory than the clinical type because of the lower range available. This thermometer was checked against a very sensitive thermometer. In observing temperatures, care was taken that the rectum was evacuated and that the thermometer was inserted to the same mark each time. The end of the bulb was inserted 39 mm. from the opening. This gave a better indication of internal temperature than would a short insertion but it is colonic not rectal temperature. Struggling was avoided because the animals were handled gently and they were accustomed to daily manipulation.

A cold room was not available, therefore the rats were exposed out of doors in a large metal box which excluded drafts of air. From six to ten rats were exposed in a wire cage 28 by 38 cm. They were in no way insulated from the metal. Unfortunately the temperatures were limited by the weather.

Metabolic determinations were made by the Haldane open circuit method, a glass desiccator serving as the animal chamber. The periods were four hours long for single rats and two hours or more for groups of three.

All adrenalectomized rats were examined for accessory cortical bodies at death. Those that did not die from exposure to cold were sacrificed a few days later for examination.

EXPERIMENTAL. In the first experiment seven rats 86 days of age, weighing 130 to 170 grams, were used. Their adrenals had been removed 31 days before. Four had been injected twice daily with cortical extracts (0.5 cc. each time) while three had been as frequently injected with an equal volume of isotonic NaCl solution. Four normal rats weighing from 85 to 106 grams were used as controls. All were subjected to the same temperature changes. At first the temperature was 14°C., falling gradually to 6.5°C. in 7½ hours. The average temperature of the rats at 27°C. was: normals, 38.2°; cortin-treated, 38.0°; NaCl-treated, 37.4°. After 4.5 hours in the cold the temperature had fallen to: normals, 37.4°; cortin-treated, 37.0°; and NaCl-treated 35.8°. After 7.5 hours in the cold, there was no change in the normals but the temperature of the cortin-treated was 34.7° and the average for two of the NaCl-treated was 26.8°, the third possessing a small accessory had a temperature of 35.7°. After four hours in the room at 27°C. the temperature of all rats had risen to within 0.2° of the original figure.

From these results it seemed very evident that untreated adrenalectomized rats could not maintain their body temperature many hours in the cold while adrenalectomized rats treated with cortin maintained their temperature far better. A larger series of animals was required to make the work more conclusive.

Two series of rats were completely adrenalectomized at one operation. The rats in series A (20 rats) were adrenalectomized 10 to 25 days before the experiment with cold. These were protected by the injection of cortical extract twice daily (the product from 15 to 25 gm. of cortex each time). The rats in series B (19 rats) were adrenalectomized 4 to 25 days before the experiment. These were injected with the same volume of isotonic NaCl solution at the same time that the cortical extract was given those in series A.

One extra injection of 0.5 cc. of the respective solutions was administered 12 hours before starting the test with cold, and others as follows: 4 hours before (0.5 cc.), at the test (0.4 cc.), and 4 hours after starting the test (0.5 cc.).

The rats of both series together with 6 normal rats of the same weight were exposed to a temperature which gradually fell from 13° to 8°C. in 10 hours, when they were again placed in the warm room (27°C.).

The average changes in colonic temperatures are shown in the accompanying chart (chart 1).

Throughout the exposure to cold the lowest temperature shown by a normal rat was 35.6°C. while one cortin-treated rat fell as low as 30°C. although this was far from typical because the next lowest was 34.3°C.

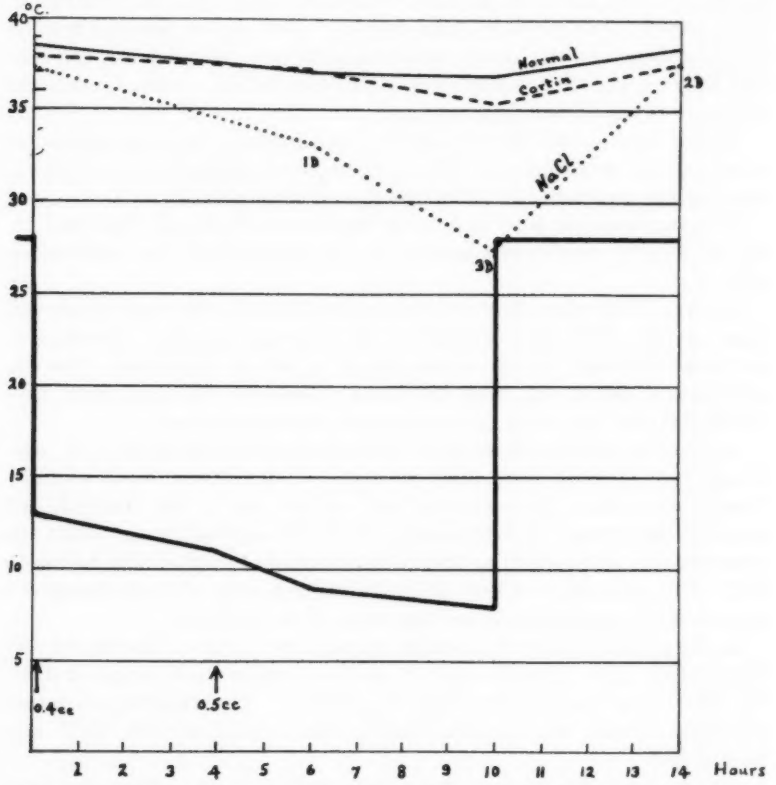

Chart 1. Changes in colonic temperature of rats exposed to cold. Heavy line, environmental temperature. Light line, normal rats. Broken line, cortin-treated adrenalectomized rats. Dotted line, NaCl-treated adrenalectomized rats.

Of the NaCl-treated rats one died at the sixth hour, three by the tenth hour and two more a few hours later. The first four were not included in making the temperature average.

When placed in the warm room again the body temperature rose very slowly in certain individuals even when extra heat was furnished.

Upon exposure to cold all rats become very active. The normals con-

tinue their activity throughout the exposure while the cortin-treated, adrenalectomized rats show somewhat decreased activity after a time and the NaCl-treated adrenalectomized rats very soon begin to crowd together and become quiet. As time goes on the adrenalectomized rats not given cortin may straggle apart and become very lethargic. These rats do not curl up in a compact ball but tend to lie with the head partially extended. The normal rats shiver in varying degrees. This can be detected best by grasping them with the hand. Shivering appears to be much less common and less vigorous than in cats or dogs that are chilled. Adrenalectomized rats seem to shiver less than normals.

It has been shown that epinephrin can increase heat production as related in the introduction. What can injected adrenalin do toward increasing the resistance of adrenalectomized rats to cold?

We compared cortin-treated with NaCl-treated animals that had received a small amount of adrenalin at the beginning of the exposure to cold.

Six rats of the same litter were adrenalectomized at 58 days of age and then injected twice daily with 0.5 cc. of a cortical extract. Levulose (1 per cent) had been added to this extract to prevent oxidation. The extract was a month old when first used. However, the last three days before the cold test, fresh cortical extract was administered.

Six rats of another litter were adrenalectomized at 64 days of age. These were injected twice daily with 0.5 cc. of isotonic NaCl solution. Twelve days after adrenalectomy both groups (one of the NaCl-treated rats had died) together with another litter of 6 normal rats of about the same age were exposed to an initial temperature of 9° gradually falling to 5°C. One injecton of 0.5 cc. of 1:100,000 adrenalin chloride was given to each NaCl-treated rat at the beginning of the cold test.

At the end of 3 hours the average temperature of the cortin-treated rats was 35.7°C. (table 1) while that of the NaCl-treated still living (2 died) was 25.7°C. and that of the normals was 38.0°C. The temperatures before exposure to cold were: cortin-treated, 38.2°; NaCl-treated, 38.2° and normal 38.9°C.

All rats except the two mentioned made good recovery. Four days later the same rats were again placed in the cold for three hours. Neither cortical extract nor isotonic NaCl had been given since the last experiment. The temperatures in both tests are shown in table 1. All animals including the normals now had a lower temperature at 27°C.

When exposed to cold the second time the rats of the "cortin" series showed a marked fall in temperature with the exception of two, one of which possessed a large accessory cortical body. The "NaCl" rats that survived the last cold test now showed greater resistance to cold.

The lower temperatures of the normal rats four days after the first cold

test are difficult to explain. Other normal rats have shown similar reductions in temperature.

TABLE 1

Temperature of rats—adrenalectomized

NO.	CORTIN-TREATED			
	At 27°C.	After 3 hours at 9°–5°C.	Four days after Cortin discontinued	
			At 27°C.	After 3 hours at 11°–8°C.
	°C.	°C.	°C.	°C.
285	39.0	35.3	37.6	23.0
286	38.1	37.3	37.3	37.0
287	38.3	35.9	37.7	31.0
288	37.7	35.2	37.3	20.2
289	38.0	38.9	37.6	38.7*
290	38.0	31.3	37.0	23.0
Average.....	38.18	35.65	37.4	28.8
	NaCl-TREATED			
	At 27°C.	After 3 hours at 9°–5°C.	Four days after NaCl discontinued	
			At 27°C.	After 3 hours at 11°–8°C.
	°C.	°C.	°C.	°C.
291	38.7	29.5	37.7	33.0
294	38.0	22.7	37.3	36.0
295	38.1	25.0	37.4	31.0
Average.....	38.2	25.7	37.46	33.3
	NORMAL			
	At 27°C.	After 3 hours at 9°–5°C.	Four days later	
			At 27°C.	After 3 hours at 11°–8°C.
	°C.	°C.	°C.	°C.
297	38.7	38.0	37.3	37.6
298	38.4	37.4	38.0	38.0
299	39.3	38.0	37.2	36.6
300	39.3	38.3	38.2	38.3
301	39.0	38.2	38.5	36.7
302	38.7	38.0	38.1	38.0
Average.....	38.9	38.0	37.9	37.53

* Large accessory

Another series of 9 rats varying in weight from 95 to 138 grams and adrenalectomized 8 days before the test, were not injected until the time

of the exposure to cold when single doses of adrenalin chloride varying from 0.05 to 2.5 cc. of 1:100,000 were given subcutaneously. After three hours' exposure to a temperature of 13° to 11°C., the average temperature was 32.0° as compared with 38.3°C. at the start. At the end of an additional 3 hour period (11 to 8°C.) five rats had died and the average temperature of those remaining was 23.9°C. In the course of several hours after the experiment all but one of these animals died. This is much less resistance to cold than is usually shown by adrenalectomized, NaCl-treated rats. It may possibly be explained by the absence of fluid injections twice daily. The injection of adrenalin at the beginning of the experiment could scarcely account for the difference because those animals that received only 0.05 cc. of 1:100,000 adrenalin fared as poorly as those receiving fifty times as much. So far our observations indicate that injected adrenalin does not protect against cold.

TABLE 2

Average heat production before and after adrenalectomy

"NaCl" RATS	BEFORE ADRENALECTOMY	DETERMINATIONS	AFTER ADRENALECTOMY	DETERMINATIONS	PER CENT CHANGE
	cal. per square meter per hour		*cal. per square meter per hour*		
227	37.07	7	27.77	3	−24
229	36.43	4	30.52	3	−16
233	32.39	5	27.88	3	−12
"CORTIN" RATS					
226	36.01	4	38.76	4	+7.6
231	33.31	5	32.63	3	−2
232	34.63	5	32.61	3	−5.8

Is the lowered resistance to cold after adrenalectomy due to greater heat loss or to inadequate heat production? The metabolism has been studied in an attempt to answer this question.

Heat production at 28°C. The metabolism of six rats was determined individually. The surface area was calculated by means of the Meeh formula, $S = KW\ 2/3$, using Diack's constant for normal rats (7.47). The animals were deprived of food 17 hours before the test began; and all determinations were made at approximately 28°C. because Benedict and Macleod found this to be the optimum temperature for basal conditions. The observations were repeated two or three times a week until fairly good checks were obtained. The adrenals were then removed. Immediately after the operation three rats were injected with 0.5 cc. of cortical extract twice daily while three rats were injected with similar volumes of isotonic NaCl solution at the same time.

The metabolism of the adrenalectomized, NaCl-treated rats fell (table 2) within two days after adrenalectomy. These died 13, 15, and 22 days after the operation. The long periods of fasting for metabolism (21 hours total at each determination) seemed to increase their cortin insufficiency.

Later, (after 17, 24 and 27 days of treatment with cortin) isotonic NaCl was substituted for cortical extract in the "cortin" rats. The metabolism fell and rats 226 and 231 died 8 and 9 days later.

Metabolism determinations were also made collectively on rats in groups of two and three of the same age. The animals were separated by aluminum partitions in the same container. The slight mutual heating of the rats did not lower the metabolism.

TABLE 3

Heat production at different temperatures

	RATS	SURROUNDING TEMPERATURE	RECTAL TEMPERATURE AT FINISH	CAL. PER SQUARE METER PER HOUR	CAL. PER SQUARE METER PER HOUR AT 28°C.	PER CENT CHANGE
			°C.			
I	9 normal	18°C. for 4 hours	36.1	54.9	35.7	53.8
II	3 adrenalectomized, NaCl-treated	16°C. for 4 hours	24.3	47.6	39.6	17
III	12 normal	19°C. for 4 hours*	36.9	54.0	39.0	38.4
IV	6 adrenalectomized, cortin-treated	19°C. for 4 hours	34.5	53.6	40.0	34.0
V	5 adrenalectomized, NaCl-treated	19°C. for 4 hours	24.9	26.6	39.0	31.8

* These rats were subjected to 8°C. for 3 hours just preceding the metabolism period.

A typical group of three rats (metabolism, 36.0 cal. per sq. m. per hr.) was followed for 22 days after adrenalectomy. Cortin was injected for 12 days, discontinued for eleven days and then started again. Metabolism remained normal (37.14 cal. 60 hr. after adrenalectomy) while cortin was injected. It dropped after cortin was discontinued (28.58 cal., 4 days and 27.42 cal., 7 days after cortin was stopped) but rose again on renewal of cortin (36.12 cal. 60 hours after injection started). Within a few days after adrenalectomy metabolism drops often 10–20 per cent. From then on there is a gradual fall until just before death when there is a more abrupt decrease. That this is due to cortin insufficiency is shown by the fact that administration of cortin after metabolism has fallen will bring it back to normal. However a large number of rats should be studied before final conclusions can be reached.

Heat production in the cold. Metabolism determinations were made upon animals exposed to cold before and after adrenalectomy (see table 3). These determinations were made after a 12-hour fast. The results are averaged for each type of animal. If the metabolism is determined during the whole period of exposure to cold the heat production is greater than when only the latter part of the cold period is used for the determination. (Groups I and II include the whole period of cold while groups III, IV and V constitute only the latter part of the cold period.) Heat production for the same animals at 28°C. and with intact adrenals is given for comparison.

The average increased heat production in a temperature of 18°C. for normal rats was 53.8 per cent while the average increase for NaCl-treated rats five days after adrenalectomy was 17 per cent.

After a preliminary cooling period of three hours at 8°C. a four hour period for metabolism at 19°C. gave an increase of 38 per cent for normal rats, 34 per cent increase for cortin-treated adrenalectomized rats and a decrease of 31.8 per cent for NaCl-treated adrenalectomized rats.

From these results it seems that heat production in response to cold in cortin insufficient rats, although it increases at first, falls far short of making up for the heat loss and later it goes below that of normal rats at 28°C. If cortin has been administered the rats react almost as well as normals.

Blood sugar after exposure to cold. Low blood sugar is frequently found in the late stages of cortical insufficiency. Therefore, it was thought that this might account for the lowered metabolism of adrenalectomized animals exposed to cold. We have determined the blood sugar in rats after cooling. Blood was obtained by heart puncture and the sugar was determined by the Hagedorn-Jensen method. Four normal rats and 26 adrenalectomized rats were exposed to a temperature of 9°C. for 4.7 hours.

At the end of the exposure to cold, blood and colonic temperatures were taken with the following results: normal—temperatures 37.0 to 38.0°C., blood sugar 99 to 122 mgm. per 100 cc.; adrenalectomized—23.0 to 37.3°C., blood sugars 36 to 130 mgm. per 100 cc. Low temperatures and low sugars did not go together. The following are representative findings: 23.0°–36 mgm., 26.0°–130 mgm., 35.0°–104 mgm., 37.0°C.–63 mgm.

From these results it is evident that there is no definite relation between low temperature and low blood sugar under the conditions of the experiment.

Reaction to cold as a test for the potency of cortin extracts. Exposure to cold increases the need for cortin. We have shown above that the temperature falls markedly and some animals die when an adequate supply of cortin is not available. Young adrenalectomized rats grow almost as fast as normals with two injections of 0.5 cc. cortin daily. Reduction in the amount of cortin leads to failure in growth but the animals do not die un-

less the reduction is too large. When testing different extracts, if growth failed it could not be determined whether this was due to the presence of toxic substances in the extract or to an absence of cortin. If cortin is present in an extract whether in sufficient quantity to produce growth or not, adrenalectomized rats treated with it show a greater resistance to cold. This can be used as a basis for testing the potency of cortin extracts. As an illustration we wished to compare four extracts: A, B, C, and D. Six-

TABLE 4

Effect of cold on adrenal weight

GROUP	EXPOSURE TO COLD		TIME SINCE EXPOSURE	WEIGHT OF ADRENALS (AVERAGE IN MILLIGRAMS)		PER CENT CHANGE	
				Controls	Cooled rats	On absolute weight	On body weight
	hours	*in days*	*days*				
I	20	3	0.6	32.26	30.26	6.2 loss	3.08 loss
II	20	3	0.6	24.16	34.4	*42.0 gain*	*27.8 gain*
III	20	3	0.6	18.2	18.7	*2.7 gain*	13.4 loss
IV	20	3	0.6	19.96	22	*10.2 gain*	*9.2 gain*
V	20	3	0.6	27.26	29.23	*7.2 gain*	0
VI	20	3	0.6	19.26	21.7	*12.7 gain*	*8.0 gain*
Average						11.2 gain	4.7 gain
VII	53	11	1	15.8*	17.3*	*9.5 gain*	0
VIII	83	27	1	13.2*	14.9*	*12.8 gain*	*11.5 gain*
IX	30	9	5	26	29.9	*11.5 gain*	*8.3 gain*
X	75	14	5	24.5	27.8	*13.3 gain*	*55 gain*
XI	30	9	5	17*	21.6*	*27 gain*	*23.5 gain*
XII	66	14	7	24.3	24.9	*2.4 gain*	7.5 loss
XIII	66	14	7	19.26	21.3	*10.6 gain*	2.2 loss
XIV	66	14	7	17.6	17.16	2.5 loss	10.9 loss
Average						10.5 gain	9.7 gain
Grand average						10.9 gain	7.6 gain

* Without capsule.

teen rats of the same size were divided into groups of four for each extract. Equal volumes of the respective extracts were injected twice daily into the corresponding group. At the end of four days all animals were exposed to the same temperature (20–12°C.) for seven hours. At the end of the period the temperatures were taken and averaged. The averages were: A, 26.0°; B, 28.0°; C, 35.0°; D, 29.9°. Extract C, therefore, was much more potent than the others, yet it had not been potent enough to maintain the growth curve. The resistance to cold is a good test to com-

bine with the growth test especially when there is insufficient hormone to maintain growth although the animal survives. We have used this test several times to check the potency of adrenal preparations.

Effect of cold on weight of adrenals. If exposure to cold increases the demand for cortin the adrenal cortex would need to become more active. This would appear to be particularly true because very little cortin seems to be stored there. Increased activity over a period of time might lead to hypertrophy.

We have compared the weights of the adrenals of 42 rats exposed to cold with those of an equal number of unexposed animals. The 84 rats were divided into sets of six of the same age and sex. Three rats of each set were exposed to temperatures ranging from 5° to 20C°. for five to six hours at a time, over a period of days or weeks until a total of 20 or more hours had been reached. The remaining three rats of each set were kept at 27° to 28°C. at all times. At intervals after the last exposure to cold the adrenals were removed, carefully dissected free from fat and weighed. The average weight of the adrenals from all rats exposed to cold was definitely greater than that for the controls (table 4). Based on absolute weight the adrenals of the rats exposed to cold averaged 10.9 per cent heavier than those of the controls. If the ratios of adrenal weight to body weight (in per cent) are compared, the difference is not quite so marked, showing an average gain of 7.6 per cent. The average gain (based on adrenal-body ratio) in animals exposed only 20 hours to cold is 4.7 per cent, while those exposed 30 hours or more showed 9.7 per cent gain. This difference was shown in spite of the fact that 18 of the 24 rats had not been exposed to cold for five days or more preceding the weighing of the adrenals. These differences appear to be too great to be accidental. We do not know whether this increase is in the cortex or medulla or both.

Discussion. The temperatures of our normal rats are somewhat higher than those given by other observers (Donaldson). This is accounted for in part by the depth of insertion of the thermometer and the high temperature in which the rats lived (27–28°C.).

Wyman and Tum Suden did not obtain nearly so marked a fall in temperature when their adrenalectomized rats were exposed to cold. These differences are probably due to their use of larger animals and shorter periods of exposure (2 hours). We have found the greatest change to occur after two hours in the cold.

It is well known that heat production depends largely upon muscular activity. Upon exposure to cold there are two easily visible means of increasing heat production, viz., voluntary activity and shivering. In the rat the latter is a weak reaction as compared with other animals and only perceptible on contact with the individual.

There is considerable voluntary activity on the part of animals that

react well to cold such as normals and to a lesser extent cortin-treated adrenalectomized animals. In untreated adrenalectomized animals this activity on exposure is greatly decreased and in the late stages becomes almost absent.

The injection of adrenalin under the conditions of our experiments did not increase the resistance of adrenalectomized rats to cold. Cortin on the other hand has a marked effect.

However, long exposure to cold does produce a greater temperature fall in cortin-treated adrenalectomized rats than in normal rats. It might be attributed to the lack of epinephrin but it could just as well be due to an inadequate supply of cortin. We have no means of determining whether cortin is being supplied as it would be by the intact adrenal. The same might be said of adrenalin injections but it has not yet been shown that they are of any value in protection against cold.

Cortin enables adrenalectomized animals to produce the extra heat required on exposure to cold. This might be explained in part by the more nearly normal muscular activity of such animals, since in untreated adrenalectomized animals the muscular activity is greatly decreased (Durant) even at room temperature, and this difference is exaggerated as the temperature falls, as pointed out above.

SUMMARY

1. A study has been made of adrenalectomized rats exposed to cold after the injection of cortin, adrenalin chloride and isotonic NaCl solution. Animals which resist cold well show considerable voluntary movement while those which do not are very quiet.

2. Normal rats show a fall in colonic temperature of a little more than 1°C. when exposed to cold (4–18°C.) for several hours. Cortin-treated adrenalectomized rats show a drop of 2° to 3° C. while NaCl-treated adrenalectomized rats show a fall of 12°C. or more and many die. Injected adrenalin did not increase the resistance of adrenalectomized rats.

3. The metabolism of rats is lowered 10 to 20 per cent by adrenalectomy but returns to normal when an adequate supply of cortin is furnished. The fall in temperature of adrenalectomized rats when exposed to cold is due to a decrease in heat production. Although the latter increases at first it falls far short of making up for the heat loss and later goes below that of normal rats at 28°C. The administration of cortin enables such animals to produce heat almost as well as normals.

4. The ability of cortical extracts to protect adrenalectomized rats against cold has been used as a test for potency.

5. There is an increase in the weight of the adrenals of normal rats exposed to cold for 20 hours or longer.

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PUPILLARY DILATATION PRODUCED BY DIRECT STIMULATION OF THE TEGMENTUM OF THE BRAIN STEM

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A center for the reflex dilatation of the pupil has been located by Karplus and Kreidl in the hypothalamus and, more specifically, in the medial and rostral part of the subthalamic nucleus. They have found that direct faradic stimulation of this region in cats produced the same effect on the eye as a stimulus applied to the cervical sympathetic trunk. Their conclusions have been favorably received (Greving, 1930; Spiegel, 1928); however, for the interpretation of their results it is important to know that in cats with intact forebrains dilatation of the pupil can be elicited by weak faradic stimulation of many other parts of the brain stem.

For this work we have used the Horsley-Clarke stereotaxic instrument, with which it is possible to stimulate one point after another in the interior of the brain, always being aware of the location of each region stimulated with reference to the others and also with reference to a fixed zero point. We have further been able to check microscopically the location of each point stimulated. The apparatus has been described by Horsley and Clarke (1908), and the manner in which we have employed it has been given in detail in a paper dealing with the limb and trunk movements elicited by stimulation of the tegmentum (Ingram, Ranson, Hannett, Zeiss and Terwilliger, 1931).

The bipolar needle, which we inserted into the brain, was made of enamelled nichrome wires which when cemented together and reinsulated with chlorinated rubber gave a total diameter of less than 1 mm. The faradic current was derived from a Harvard inductorium with a primary current of 1.5 amperes, the secondary coil being set at 13 cm. and 45°. By actual observation it was found that this gave a stimulus at or just below the threshold of the fibers of the pyramidal tract when the needle was in the basis pedunculi.

OBSERVATIONS. The analysis of the results as recorded in the protocols of the various experiments was carried out by tabulating the responses obtained with reference to the different areas stimulated. By far the most widespread and consistent reaction to stimuli of the low intensity employed was found to be dilatation of the pupils, and with this phenomenon the pres-

ent paper will deal exclusively. It was at once observed that dilatation of the pupils could be elicited from many more regions than one can infer from the work of Karplus and Kreidl. In certain cases the hypothalamus, subthalamus and extreme ventral part of the thalamus were explored as far rostrally as the pre-optic area. In many others the entire tegmentum of the mid-brain and pons was explored, in some of these as far caudally as the trapezoid body. The terminology used in the following description is that of Rioch (1929) and Papez (1929).

The periventricular gray of the more rostral levels produced bilateral dilatation of the pupils in two of three cases; the other cat, although giving no pupillary response, gave retraction of the nictitating membranes. Dilatation was elicited from the nucleus hypothalamicus dorsomedialis in two out of three cases, and from the nucleus hypothalamicus ventromedialis in one. The lateral hypothalamic area, which constitutes a large part of the region designated by Malone (1910, 1914) as the nucleus mammillo-infundibularis, gave dilatation in each of four cats. Certain parts of this region, irregularly distributed, failed to give this response; these are of interest because of the frequency with which elevation of the nictitating membranes occurred at these points, retraction of the former being associated with dilatation, usually. Two cats in which the nucleus hypothalamicus posterior was stimulated gave dilatation of the pupils, but three cases where the external medullary lamina was involved gave negative results. Dilatation was rarely secured from the extreme ventral part of the nucleus ventralis proper; the nucleus submedius was negative, while only one case of slight dilatation was obtained from three cats in which the nucleus subparafascicularis was stimulated. In two cats the supramammillary nucleus was stimulated, in each case producing slight dilatation. The medial and lateral mammillary nuclei may rarely give this response, although it was not obtainable from every point stimulated within these nuclei. One would hardly expect consistent positive results from these cell groups, which are thought to be related chiefly to olfactory reactions.

The zona incerta, in four cases, showed many points at which dilatation could be obtained, and the H_2 field (3 cases) gave a number of positive responses. The optic tract was stimulated in only two cases, the results indicated a tendency to elevation of the nictitating membranes, while one, with the weak stimuli employed, gave bilateral constriction of the pupils. The H_1 field, with the rostrally extending fibers of the anterior capsule of the red nucleus, gave positive results in each of 14 cases. The corpus subthalamicum produced dilatation in three of five cases, some tendency to widening of the palpebral fissures also being observed. This is of some interest in view of Karplus and Kreidl's designation of this nucleus as the hypothalamic center for sympathetic reactions. In our cases, however, the dilatation, which was not especially strong, was elicited from points farther

lateral than the focus found by these workers. Fibers of the spino-tectal system were stimulated in twenty-four cats, seventeen of which produced dilatation of the pupils in varying degrees. It must be pointed out, however, that in many of these cases other fiber areas and cell groups belonging to the tegmentum were stimulated at the same time. Stimuli within and at the borders of the red nucleus (6 cats) gave positive pupillary responses, frequently along with retractions of the nictitating membranes. The central tegmental fasciculus region gave dilatation in all of the twenty-two cats in which it was stimulated, and the central gray of the mesencephalic region gave similar responses, associated with retraction of the nictitating membranes and widening of the palpebral fissures in nine of eleven cats. Eleven of fourteen cats in which the needle reached the cerebral peduncle also gave varying degrees of bilateral dilatation, although the stimuli used were not sufficiently strong to arouse typical pyramidal muscular responses in some cases.

The reticular formation of the mesencephalon and pons was extensively explored in a large series of cats. In nineteen of these, in which the points stimulated were identified microscopically, dilatations were found to be obtainable from practically anywhere in the reticular formation of the tegmentum. These responses were all bilateral, and most of them of a maximal degree, although occasionally a weak reaction was observed. This area includes a number of fiber tracts in transit, among these the brachium conjunctivum gave consistent dilatations in all but one of the fourteen cases in which it was found to have been stimulated. However, the response could not be obtained from this tract at its more dorso-caudal levels, before it entered the reticular formation. The medial lemniscus also yielded consistently positive results, as did the spino-thalamic tract. From the latter region characteristic cries were frequently evoked, but it is difficult to draw specific conclusions from this because other areas also occasionally produced cries. From the region of the rubro-spinal tract a large number of strong dilatations were elicited in every cat in which this area was known to have been stimulated. Instead, however, of a tendency to widen the palpebral fissures, the stimulation of the rubro-spinal tract in many cases caused contraction of the homolateral orbicularis oculi muscle.

Four cats in which the medial longitudinal fasciculus was stimulated gave in each instance dilatation of the pupils, widening of the palpebral fissures, and in two of these, retraction of the nictitating membranes. The lateral lemniscus region, which may well have included fibers belonging to other systems, gave slight dilatation in two cases; while the inferior colliculus and its brachium were essentially silent. The substantia nigra gave positive results in varying degrees in eleven of fourteen cats. There was some possibility, however, that fibers of the peduncle or the ventral part of the reticular formation may have been stimulated.

Pupillary dilatation resulting from the stimulation of other parts of the brain than the corpus subthalamicum have been noted by Shinosaki (1929), Herzfeld, Kroner, Krüger, and Lichie (1929), and Spiegel and Takano (1929). Lewy (1928) and Shinosaki (1929) obtained varying responses from the corpus subthalamicum, depending apparently on the character and strength of the stimulus, and their results are difficult to account for except on the basis of a spread of current. Lesions in the ventral part of the diencephalon are said to increase the irritability of the cervical sympathetic (Herzfeld, Kroner and Krüger, 1929; and Herzfeld and Liu, 1928).

While the results of Karplus and Kreidl point to the existence of a specific isolated center for pupillary dilatation in response both to pain impulses from the periphery and to direct stimulation, it does not seem that their experiments give adequate evidence that this response cannot be obtained by the stimulation of other regions, and it is clear from the observations reported in this paper that in animals with intact forebrains pupillary dilatation can be obtained from many parts of the brain stem. The response is most easily and most regularly elicited from the tegmentum, which is composed of reticular substance in which many tracts are imbedded. While it can be obtained from the regions occupied by these tracts we have found no evidence that any of the latter are specifically concerned. In this connection it is interesting that stimulation of the region occupied by the brachium conjunctivum causes dilatation of the pupils, but if this bundle is stimulated before it enters the tegmentum no response is forthcoming.

That our observations are complicated by escape of current seems to us extremely improbable in view of the use of bipolar electrodes and very weak current. Further, in our experiments we have noted the sudden appearance of contralateral motor responses as the needle entered the pyramidal tracts. If there had been significant escape one might expect such an occurrence while the electrodes were still at some distance from the peduncle. It may also be noted that constriction of the pupils, aside from that already mentioned, occurred when the needle was in the vicinity of the oculomotor nucleus.

Although the corpus subthalamicum may play a part in such responses as have been discussed, it seems clear that in the intact brain these responses may be elicited from a wider area. Whether one can account for this, in part at least, by hypothecating the activation of fibers which by their ascent to higher centers, or otherwise, may evoke inhibitory impulses acting upon the Edinger-Westphal nucleus, whether the center for these reactions is a diffuse one, or whether descending pathways are activated by direct stimulation cannot be determined from our observations. If the latter alternative is true the descending path must consist of widely scattered fibers. Huet (1911) has advanced the opinion that there is no ciliary center

in the diencephalon, but that direct stimulation in the central nervous system brings into activity a wide system of converging fibers which carry impulses that eventually leave the central nervous system over the cervical sympathetic chain.

SUMMARY

With the Horsley-Clarke stereotaxic instrument and bipolar needle electrodes various points in the brain stem and ventral part of the diencephalon were stimulated with a faradic current of low intensity. Dilatation of the pupils could be obtained from many regions besides the subthalamic nucleus. It could be obtained regularly from all points in the tegmentum of the mesencephalon and pons.

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THE BASAL METABOLISM OF OKLAHOMA WOMEN[1]

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In March, 1930, during the process of selecting physically normal college women for a feeding experiment, the question of what constitutes normal basal metabolic rates for Oklahoma women presented itself. Some fifty girls, volunteers from nutrition and hygiene classes, were tested, but the results were not comparable to those observed by the author using similar technique with girls of like ages and interests from the nutrition classes at the University of Chicago. In the fall of 1930 increased laboratory facilities and coöperation with the student health service department of the college afforded the opportunity to observe many more women, particularly freshmen just in from Oklahoma farms and small towns. The aim in this paper is to present the data for 101 normal Oklahoma women, suggesting thereby tentative standards for this section of the country.

EXPERIMENTAL. *Subjects.* Of the 101 women reported in this paper 58 were native Oklahomans and had spent all of their lives in Oklahoma. The remaining ones had lived in Oklahoma or neighboring southern states longer than the last half of their lives. Their ages ranged from 17 to 36, most of them 18 to 25, and their weights fell within ± 10 per cent of the Wood Standards (cited by Rose, 1929). All cases were rejected in which physical examinations showed any abnormality likely to affect thyroid activity, or in which there was history of recent thyroid medication. Observations were also excluded if an elevated temperature during the test was recorded or if duplicate tests failed to agree within 5 per cent and were not repeated on another day. No subject was observed during menstruation nor nearer than three days to the beginning or end of the menstrual period.

During the spring of 1930 the subjects came without breakfast to the laboratory and rested one-half to three-fourths hour before the test. Beginning with September, 1930, the privilege of using a ward in the college hospital as a metabolism laboratory made it possible to require all subjects to sleep over night on the bed where they were tested before

[1] Published with the permission of the Director of the Agricultural Experiment Station, Oklahoma Agricultural and Mechanical College, Stillwater.

rising the following morning. The girls were asked to come to the hospital not later than 8:00 p.m. of the evening preceding. Case histories were taken, height and weight recorded, being later checked against physical examination data, and the girls were in bed soon after 9:00. This procedure ruled out surreptitious eating and factors of excitement such as pep meetings, ball games, dancing, and others common to college life. No observations were made during the week preceding holiday vacations, such as Thanksgiving and Christmas, nor during the week previous to or the week of final examinations. Tests on mornings preceding scheduled quizzes were avoided as far as possible. Observations of freshmen and those just entering college were made as soon after the opening of school as they could be scheduled, before the students became adjusted to new environment, the physical, social, and mental life of college as well as perhaps different food habits. This, it was believed, would give a truer picture of the basal metabolism of average Oklahoma women.

Apparatus. A new Benedict-Roth kymographic apparatus was used, and two to four six-minute tests were made during each observation period. Calculations were based on the two lowest concordant values obtained, in most cases, in the initial observation period. Pulse rates, oral temperatures, and any history of discomfort were recorded. The apparatus was tested regularly for leaks, and the soda lime for carbon dioxide absorption. Wilson soda lime was used, and records show that this had to be changed after every 24 to 28 twelve-minute tests, and that an average of 60 to 65 grams of soda lime per test was used. Because results tended to be low, every precaution was observed to assure complete absorption of carbon dioxide.

The spirometer of the apparatus was calibrated in three different ways: Measuring volume in different portions by use of water, inside diameter by calipering, and inside circumference by means of a strip of light weight cardboard. Deviations, according to the respective methods, were −0.6, −1.1, and −0.9 per cent, from the spirometer factor employed in calculation of volumes of oxygen consumed.

Results and discussion. The averages according to age groups, including comparisons with present commonly used standards, are shown in table 1. The 17 to 19 year old groups represent chiefly college freshmen soon after entering school, the 20 to 24 year old groups about half and half upper and lower classmen, while the 25 to 29 year old group is composed of junior and senior nutrition students. The 30 to 39 age group includes instructors and laboratory technicians who qualified as Oklahomans.

The calories per square meter per hour, using the height-weight formula, decrease slightly with age, excepting in the 25–29 year group to be noted later. The average for all ages, 32.6, is very close to that for any

one group. Deviation from the Du Bois (1927) standard varies from −15.5 for the youngest group to −10.6 per cent for the 25–29 age group. Although some groups are small, it will be noted that four out of the seven groups average a deviation very close to −14 per cent, −13.2 per cent being the average for all ages.

In general these women run 10 to 11 per cent below Harris-Benedict standards (Du Bois, 1927) and about the same below the Dreyer (Du Bois, 1927). The presence of the 17 year old girls in college and the probable earlier maturity of Southern women, suggest that these may represent an older group, physiologically, than the 17 year old girls for whom the standards, particularly those of Du Bois, were intended.

TABLE 1

Basal metabolism of Oklahoma women

Averages according to age group

AGE GROUP	NUMBER OF CASES	AGE		HEIGHT	WEIGHT	WEIGHT DEVIATION	SURFACE AREA	CALORIES PER 24 HOURS			CALORIES PER SQUARE METER PER HOUR	DEVIATION FROM STANDARD		
								Total	Per kgm.	Per cm.		Du Bois	Harris-Benedict	Dreyer
years		*yrs.*	*mos.*	*cm.*	*kgm.*	*per cent*	*sq.m.*					*per cent*	*per cent*	*per cent*
17	6	17	2	159.6	51.7	−3.0	1.52	1,226	23.9	7.7	33.7	−15.5	−10.0	−11.1
18	14	18	1	164.1	56.5	−2.0	1.61	1,265	22.5	7.7	32.8	−13.7	−10.6	−11.2
19	15	19	2	163.2	54.6	−2.7	1.58	1,235	22.6	7.5	32.5	−14.4	−11.1	−11.9
20	14	20	0	163.3	55.1	−3.7	1.59	1,238	22.5	7.6	32.5	−12.1	−11.5	−11.6
21–24	39	22	0	161.7	56.7	0	1.60	1,248	22.1	7.7	32.6	−11.9	−10.5	−10.6
25–29	7	25	11	164.4	57.2	−3.4	1.62	1,284	22.5	7.8	33.0	−10.6	−7.3	−6.7
30–39	6	32	6	164.5	56.9	−6.2	1.62	1,217	21.8	7.6	31.3	−14.3	−10.1	−9.0
Average								1,245	22.5	7.7	32.6	−13.2	−10.1	−10.1

The calories per square meter per hour in 100 per cent of the cases in the 17, 18, and 25–29 age groups, and in 92 per cent of the entire number fall within a range of ±10 per cent from the averages for the respective groups, whereas a ±15 per cent range includes 100 of the 101 subjects. On the other hand 86 per cent of the 18 year old group and 71 per cent of the entire number are below the −10 per cent limits of the Du Bois predictions, and 37 per cent below the −15 per cent limits. These facts emphasize the inapplicability of present standards to Southern women.

The average heat production per centimeter of height, 7.7 calories per 24 hours, is practically constant throughout, and is 9 per cent below the 8.4 for McKay's (1930) Ohio girls, 14–18 years of age, and the 8.5 for Wellesley women 18–21 years of age (Gustafson, 1928).

The average of calories per kilogram, 22.5 per 24 hours for all women, shows gradual decrease with advancing age, except in the 25–29 age group, to be noted later. Blunt (1926) reports 25.7 for 17–18 year old girls, Du Bois (1927) 25.5 for 20–29 age group, Benedict (1923) 21.8 for sleeping Girl Scouts of 17–18 years, and 23.9 for college women (Gustafson, 1928), and MacLeod (1925) 23.0 calories per kilogram per 24 hours for a New York group.

There was considerable individual variation from these averages. The lowest rate observed was −28.6 per cent with reference to the Du Bois standard, and two cases show −27 per cent. The highest rate observed was +0.5 while three persons exhibited a −1.6 per cent.

Pulse rates varied from 48 to 88, and averaged 69 per minute, and respiration varied from 8 to 22 per minute, with an average of 13.

The higher average metabolism for the 25–29 age group, though small, deserves special consideration, because of the implications made regarding

TABLE 2

Effect of group characteristics on averages for basal metabolism

	NUTRITION STUDENTS, AGES 20–29				NON-NUTRITION STUDENTS, AGES 20–29			
	Spring, 1930	Fall, 1930	Spring, 1931	Totals	Spring, 1930	Fall, 1930	Spring, 1931	Totals
Number of cases	19	7	13	39	9	26	4	39
Per cent weight deviation	−2.2	−1.1	−3.1	−2.3	−2.2	−4.2	−2.9	−3.6
Calories per square meter per hour	33.2	33.3	33.0	33.2	30.8	32.9	32.9	32.4
Per cent deviation, Du Bois prediction	−10.3	−9.9	−10.7	−10.3	−16.8	−11.1	−11.2	−12.4

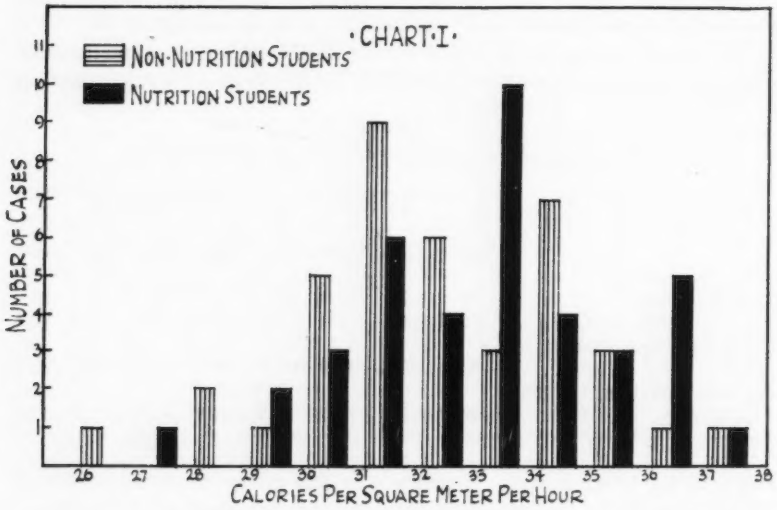
averages for other groups. Inasmuch as this group consisted wholly of nutrition students, juniors and seniors, the question arose as to whether or not this might represent a more select group than those of earlier ages. The fact that this school seeks to attract boys and girls of the less favored classes economically, providing free tuition and sponsoring a number of self-help student industries, and the fact that over 50 per cent of the freshmen women never appear in the junior and senior years, for financial and scholastic reasons, seem pertinent to the question.

Accordingly, the average calories per square meter per hour for the advanced nutrition students have been tabulated in comparison with non-nutrition students of like ages but of varied academic standing (table 2). The table includes all normal women 20 to 29 years of age observed to the present, who were within ±15 per cent of standard weight, this last condition in order to have comparable groups with respect to weight and numbers. There are 39 subjects in each group. It will be seen that the

nutrition group averaged slightly but regularly each season above the non-nutrition group, and that the average of 33.0 calories per square meter per hour for the 25–29 age group (table 3) is in close agreement with the averages for all nutrition students.

Since individual variations play a prominent part in both groups, the distribution of cases with respect to heat production per square meter per hour is graphed in chart 1, and describes the picture of higher basal metabolism more accurately.

The season of the year and the comparative weight deviations are not possible factors as table 2 shows. Although most of the nutrition group was observed in the spring and the majority of the mixed group came in



the fall, averages within groups indicate that season made no consistent difference. Nor did the change from preliminary resting periods used in the spring, 1930, to overnight sleep in the hospital used after September, 1930, seem to affect the averages obtained in different groups. The average weight is slightly lower in non-nutrition groups, but the lowest weight does not occur along with the lowest average basal metabolism in that group. On the other hand, the state of nutrition and physical development is not accurately estimated by weight alone, for a girl may measure well up to accepted weight standards but be anemic, possess flabby muscles, and lack vitality in general.

The "trained subject" cannot be said to be a factor in either group, and the advanced students had distinct advantage in respect to appreciа-

tion for conditions necessary to basal metabolism tests, yet showed a higher average rate. An average slightly older age, 22.7 compared to 21.6 years, was likewise in their favor for expected lower rates.

It will be observed that the final average of 32.4 calories per square meter per hour for the non-nutrition group is fairly well in line with the gradual decrease shown by the other age groups of mixed composition, and that the 33.2 for the nutrition group is in fair agreement with the 33.4 reported by Tilt (1930) for 52 Florida women with similar training and interests, but living in a more tropical and humid climate.

Whether the higher level of metabolism is to be regarded as that of a more select group physically and mentally, being composed of upper classmen, whether it is the consequence of a speeding up of general activity as a result of the complexities of college life, or whether the metabolism is an expression of a degree of vitality which acts as a factor affecting the choice of continued college careers, are suggestions for further study. The results emphasize the fallacy of basing standards intended for general application on observations of single homogeneous groups, and constituted the chief motive for extending this preliminary study to larger numbers rather than confining it to repeated observations on a few individuals. Investigations of the latter type are now in order and can be expected to lower slightly the tentative standards suggested in this paper.

SUMMARY

1. Figures are presented for the basal metabolism of 101 "normal" Oklahoma women varying in ages from 17 to 36 years. These average 13.2 per cent below the Du Bois and 10.1 per cent below the Harris-Benedict and the Dreyer predictions.

2. Averages for the entire group were 1245 calories per 24 hours, 32.6 calories per square meter per hour, 22.5 calories per kilogram of body weight and 7.7 calories per centimeter of height per 24 hours.

3. Junior and senior college women with training in nutrition showed slightly but rather consistently higher averages than other women of like ages and sizes, thus emphasizing the probable error from general conclusions based on observations of select groups.

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BASAL METABOLISM IN RELATION TO NUTRITIONAL STATUS[1]

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The question of whether or not undernutrition was contributing unduly to the low averages previously reported for this section of the country (Coons, 1931) and the problem of apparent increase in the numbers of undernourished girls coming for college entrance, together with the necessity for a knowledge of the basal metabolic rate before beginning corrective nutrition work on very thin or obese students, led to plans for observations of basal metabolism in underweight and overweight college women. The body weight is at best only a crude criterion of nutritional status, but in this series in the absence of more concrete standards it is sufficient to indicate general tendencies, and to aid in orienting further investigations.

LITERATURE. Recent reviews of the literature on basal metabolism in relation to undernutrition and obesity (Du Bois, 1927, 1930; Peters, 1931) obviate the necessity for much discussion of earlier reports. Wang (1926) found little change in the basal metabolism of children up to about 15 per cent underweight, except that it was high on the basis of weight alone. More recently Topper and Mulier (1929) have reported that the basal metabolism of overweight children tended to be higher than normal.

Du Bois (1927), Blunt (1926), Lusk (1928), McKay (1930), and others are fairly well agreed that the basal metabolism of persons of abnormal body build can be compared to those of average build only on a basis of calories per square meter of body surface; that for the underweight individuals the total calories are low, calories per centimeter of height low or normal, and calories per kilogram of weight high, while for overweight the reverse is true, total calories and calories per centimeter high, calories per kilogram low. On the other hand, Benedict's (1919) young men, during a prolonged period of underfeeding, showed a lowered basal metabolism whether expressed as total calories, calories per kilogram, or calories per square meter.

Boothby and Sandiford (1922) reported that 81 per cent of their cases of obesity were within the +10 to −10 percentage range, Du Bois prediction, while 9 per cent were above and 10 per cent below this range.

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EXPERIMENTAL. Figures for the basal metabolism of 38 college women with a weight variation more than +10 and 80 more than −10 per cent, according to Wood's standards (cited by Rose, 1929), are reported in this paper. The ages range from 17 to 28 years, and the weight deviations from −29 to +102 per cent. Most of the girls were of freshmen and sophomore college standing and were selected, in the course of regular physical examination, on account of their apparently abnormal state of nutrition. Others came as volunteers from nutrition and hygiene classes and similar interested groups. It is to be regretted that more overweight girls were not available for observation.

Every girl qualified as an Oklahoman, having spent all or the majority of her life in this state. All subjects for whom figures are reported were rated "normal" in the sense that no defects likely to affect basal metabolism were noted, but the possibility of incipient disease in such individuals is an ever present uncertainty. On the other hand, seven cases were excluded because of goiter symptoms or recent thyroid medication, six because X-ray or sputum examination confirmed a diagnosis of phthisis, two each on account of nephritis and diabetes, and four for various other abnormalities. It must be remembered, too, that weight in relation to age and height is only an approximate index of the state of nutrition.

The technique and apparatus employed were those described in the previous paper (Coons, 1931), most of these girls having been tested after the adoption of the over-night-in-the-hospital procedure.

RESULTS AND DISCUSSION. Table 1 summarizes by age groups the fundamental data for both over- and underweight cases, thus facilitating comparison with similar ages of average body build. The average heights are not unusual and are not far from those reported for average weight women. The weight extremes, +46.7 for one group, are greater in the overweight than in the underweight class, but the number of cases is greater in the latter. The Harris-Benedict and Dreyer predictions tend to run closer to the actual than do the Du Bois.

In general, it will be seen that the tendency is for an average heat production above normal in the overweight and below normal in the underweight by every method of comparison used, including that of calories per square meter of surface area, height-weight formula. This tendency is more evident from table 2, in which the final summaries are arranged in order of decreasing body weight.

The progressive increase in calories per kilogram and the decrease in total calories and calories per centimeter associated with lower body weight is more marked and constant than the change in calories per square meter. The evidence of these variations, even in the borderline cases between 10 and 15 per cent over- or underweight, is definite and consistent, as will be seen by following the changes in percentage variation in table 2. A range

of 25 per cent between the most over- and most underweight groups is noted with respect to total calories, calories per centimeter and calories

TABLE 1

Basal metabolism of overweight and underweight college women

Averages according to ages

NUMBER OF CASES	AGE		HEIGHT	WEIGHT	WEIGHT DEVIATION	SURFACE AREA	CALORIES				DEVIATION FROM PREDICTION		
							Per 24 hours	Per square meter per hour	Per kilogram per 24 hours	Per cubic meter per 24 hours	Du Bois	Harris-Benedict	Dreyer
Weight +15 per cent or more													
	yrs.	*mos.*	*cm.*	*kilo*	*per cent*	*sq. m.*					*per cent*	*per cent*	*per cent*
6	17	5	164.1	76.3	+33.5	1.82	1,532	35.0	20.6	9.3	−12.5	−4.9	−8.7
6	18	1	165.5	73.7	+26.2	1.82	1,448	33.1	19.7	8.7	−12.8	−8.6	−11.7
4	19	0	159.0	65.1	+20.5	1.67	1,388	34.5	21.3	8.7	−9.2	−7.3	−9.9
7	19	11	163.1	70.8	+25.4	1.76	1,334	31.5	18.9	8.1	−14.8	−12.5	−16.4
6	22	0	168.1	88.9	+46.7	1.98	1,657	34.7	18.8	9.8	−6.2	−3.6	−5.6
2	27	0	152.7	67.6	+27.0	1.65	1,421	35.9	21.0	9.3	−3.0	−2.5	−4.7
Weight +10 to +15 per cent													
2	18	0	163.8	64.4	+11.7	1.70	1,420	35.0	22.2	8.7	−7.9	−4.5	−7.1
2	19	0	161.1	61.8	+11.8	1.65	1,418	36.0	23.1	8.8	−5.4	−8.2	−7.7
2	20	0	161.3	62.2	+11.0	1.66	1,388	35.0	22.4	8.6	−5.5	−4.6	−6.5
1	24	10	161.9	64.4	+11.8	1.68	1,349	33.3	20.9	8.3	−10.0	−7.3	−8.2
Weight −10 to −15 per cent													
2	17	3	160.7	47.8	−12.4	1.48	1,107	31.2	23.2	6.9	−22.1	−16.6	−16.8
10	18	1	164.9	50.1	−12.5	1.53	1,198	32.5	23.9	7.3	−14.5	−12.2	−11.4
4	19	0	165.7	49.9	−13.0	1.54	1,182	32.0	23.6	7.1	−15.2	−12.5	−11.9
4	20	0	161.7	49.4	−12.0	1.50	1,208	33.5	24.4	7.5	−12.8	−9.1	−9.2
11	21	6	164.9	51.1	−12.1	1.55	1,229	33.0	24.0	7.4	−10.8	−8.9	−8.0
Weight −15 per cent or more													
6	17	1	162.2	46.8	−17.0	1.47	1,129	32.8	24.8	7.2	−17.9	−12.2	−10.2
8	18	1	162.8	45.4	−19.9	1.46	1,099	31.4	24.2	6.7	−17.4	−15.1	−14.8
9	19	0	163.4	46.0	−18.5	1.47	1,145	32.5	25.0	7.0	−14.5	−12.5	−10.1
5	20	0	162.9	44.4	−21.4	1.44	1,126	32.5	25.4	6.9	−12.2	−12.5	−10.3
18	21	9	160.4	44.7	−19.6	1.43	1,138	33.0	25.5	7.1	−10.7	−10.9	−8.7
3	25	8	164.3	49.1	−16.9	1.52	1,208	33.2	24.6	7.3	−10.4	−7.6	−5.3

per kilo. These findings are in accord with, but more marked than, those of Blunt (1926) and McKay (1930).

There are exceptions to these tendencies in both classes, as well as individual exceptions which vary from the normal considerably more than do group averages, and the degree of variation is not in the same proportion as the degree of over- and underweight, particularly in the obese. The behavior of individual cases can be seen from chart 1, in which the heat production for each subject, on the basis of calories per square meter, is compared to averages for Oklahoma women of normal body build. The majority of obese cases have a basal metabolic rate above the average, 23 per cent of them with more than +10 percentage deviation; while the

TABLE 2

Heat production in relation to weight deviation

WEIGHT GROUP	NUMBER OF CASES	WEIGHT DEVIATION	CALORIES				PERCENTAGE VARIATION FROM AVERAGE				VARIATION, DU BOIS
			Per 24 hours	Per square meter per hour	Per kilogram per 24 hours	Per cubic meter per 24 hours	Total calories	Per square meter	Per kilogram	Per centimeter	
Ages 17 to 19 inclusive											
		per cent									*per cent*
+15 and above	16	+27.5	1,464	34.2	20.4	8.9	+17.5	+3.3	−10.5	+17.1	−11.8
+10 to +15	4	+11.8	1,419	35.5	22.6	8.8	+13.9	+8.2	−1.0	+15.8	−6.6
−10 to +10*	35	−2.5	1,245	32.8	22.8	7.6	±0	±0	±0	±0	−14.3
−10 to −15	16	−12.6	1,183	32.2	23.7	7.2	−4.9	−1.8	+3.9	−5.2	−15.6
−15 and below	23	−18.6	1,125	32.2	24.7	6.9	−9.6	−1.8	+8.3	−9.2	−16.4
Ages 20 to 29 inclusive											
+15 and above	15	+34.1	1,475	33.4	19.1	8.9	+18.5	+2.4	−4.9	+15.7	−9.8
+10 to +15	3	+11.3	1,375	34.4	21.9	8.5	+10.4	+5.5	−1.3	+10.4	−7.0
−10 to +10*	60	−1.3	1,245	32.6	22.2	7.7	±0	±0	±0	±0	−11.8
−10 to −15	15	−12.1	1,223	33.1	24.1	7.4	−1.7	+1.5	+8.5	−3.9	−11.3
−15 and below	26	−19.6	1,144	32.9	25.1	7.1	−8.1	+0.9	+14.4	−7.8	−10.9

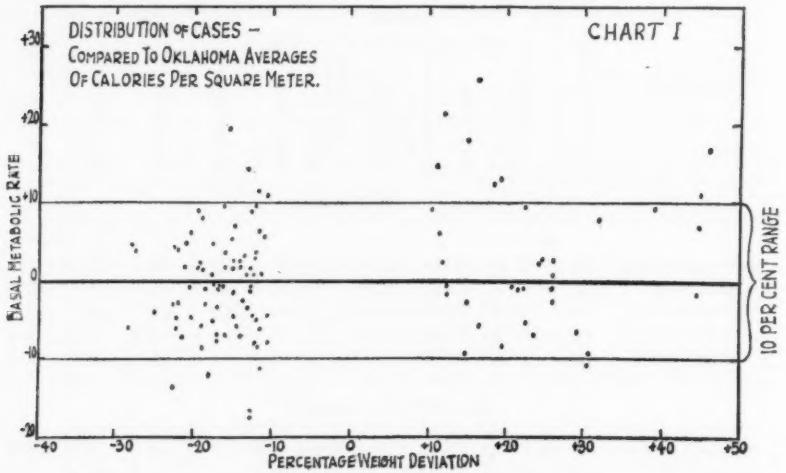
* Summarized from data reported previously (Coons, 1931).

underweight cases rank below the average line in the same respects with only 5 per cent of their number being above the +10 range. The two most overweight women, +102 and +87.6 per cent, were not easily included in chart 1, but had rates of −0.8 and −9.7 per cent, Du Bois standard, or +12.5 and +7.1 compared to Oklahoma averages.

The three lowest rates, −31.0, −27.6, and −25.7, Du Bois standards, were from girls −12.7, −12.8, and −18.0 per cent underweight respectively. The only plus rates, +7.8, +5.4, +3.9, and +2.7, were exhibited by girls with +17, −16, +11, and +46.4 per cent corresponding weight deviations.

In 84 per cent of the 118 girls the basal metabolic rate falls within the ±10, and in 94 per cent within the ±15 percentage range, of the averages per square meter shown by Oklahoma women of normal weight (chart 1). These facts indicate that the averages previously reported are probably not far from correct for college women in this section, and testify to the probable normality of most of the cases reported in this paper. They are also in accord with Boothby and Sandiford's (1922) findings for 94 obese cases, 81 per cent of whom were within the ±10 percentage range of normals for that section.

The results reported in this and the previous paper suggest that the lower averages reported for these Oklahoma women may be influenced, partly, by



the state of nutrition of the subjects, and hint that the present day tendency for lower food consumption may be contributing to the slightly lower metabolic rates being reported for women (Du Bois, 1930), since the effect of the training of subjects, a factor discussed by Du Bois, cannot be said to enter into the data reported in these two papers.

Whether the effect of nutritional status on basal metabolism may be considered as slight as these average results indicate, or whether the method of selecting subjects has included in each class some in whom factors other than dietary were operating to affect the basal metabolic rate in the opposite direction, are questions to be considered.

SUMMARY

1. Data on 38 overweight and 80 underweight college women, ages 17 to 28, with weight extremes −29 to +102 per cent, are reported.

2. The overweight women tended to metabolize at a higher rate, the underweight at a lower rate, whether expressed as total calories, calories per centimeter, or calories per square meter; and the reverse was true when heat production was expressed as calories per kilogram.

3. The effect of undernutrition and overnutrition tended to manifest itself even when the weight variations were slight, between 10 and 15 per cent above or below the average. The magnitude of the basal metabolism deviation tended to increase with the weight variation, but not in the same proportion.

4. The basal metabolic rates in 84 per cent of these cases were within the ± 10 percentage range, 94 per cent within the ± 15 when compared to averages per square meter for Oklahoma women.

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CAPILLARY PRESSURE AND HYPEREMIA IN MUSCLE AND SKIN OF THE FROG

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In an earlier study of capillary blood pressure in the mesentery of the frog (Landis, 1926) it was observed that a spontaneous increase in the velocity of blood flow was accompanied by a simultaneous rise of capillary pressure. Moreover, with the ordinary circulation of the exposed mesentery the fall in pressure was located chiefly in the finer arterioles and to a less extent in the capillaries. On account of these findings it was suggested that the peripheral resistance to blood flow is not limited to the arterioles alone but depends in part on the tone of the capillary blood vessels.

The frog's mesentery, as is pointed out by Krogh (1929), presents rather special circulatory conditions: in the first place because the mesenteric veins belong to a portal system in which the blood meets a secondary resistance in the liver, and in the second place, because the mesenteric capillaries are rather longer than those found elsewhere. In the observations here described the pressure measurements have been extended to include the more representative capillaries of muscle and skin during normal blood flow and during hyperemia. From these additional determinations it appears that the level of capillary pressure is approximately the same in mesentery, muscle, and skin and that rapid capillary flow in each of these tissues is accompanied by a significant rise in capillary pressure.

MATERIAL AND METHODS. The observations were all carried out on brown frogs (Rana temporaria) during the months of October and November. For the muscle capillary studies urethrane was injected in the dose of 0.5 cc. of 25 per cent solution into the thigh muscles or intraperitoneally in order to avoid direct contact between the concentrated urethane solution and the muscle used for the capillary pressure observations.

At first the submaxillary muscle in the base of the mouth was examined but this tissue could be fixed only by stretching it over a moulded wax support placed in the mouth. Even this degree of tension, however, was sufficient to produce venous congestion as indicated by the abnormally high pressure in the smaller venules and veins. The most suitable location proved to be the anterior end of the M. longissimus dorsi, which is held

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quite firmly in its natural position by many attachments to the cranium and the spinal column. To expose this muscle the animal was laid on its ventral surface and a midline incision was made through the skin of the back. The vertebral border of the scapula was then caught in forceps and lifted to put tension on the muscles and fascia running from the scapula to the spinous processes of the vertebrae. These tissues were divided as close to the midline as possible. Then the anterior and posterior borders of the scapula were freed by cutting the rhomboideus muscles. With fine scissors the mesial 3 to 4 mm. of the scapula were cut away, avoiding the rather vascular attachments of the ventral scapular muscles. This exposed the most anterior portion of the M. longissimus dorsi without manipulating the muscle itself in any way.

Reflected light was used for illumination, the rays from a 6 volt 5 ampere lamp being filtered to remove heat and passed through a green screen to heighten contrast. The surface of the muscle was bathed in a continuous flow of Ringer's solution, the pH of which was adjusted to between 7.2 and 7.6. Capillary pressure was measured by the micro-injection method previously described in detail (Landis, 1926), though slight modifications were necessary because of differences in the arrangements of the capillaries in muscle and skin as compared to mesentery.

In the longissimus dorsi muscle the arteries and veins are concealed beneath the surface except in the zone where the muscle fibers insert into the tendon. The capillaries which are visible on the surface originate in groups of two to four from vertically placed arterioles, the terminal divisions of which are the only parts to be seen, since each arteriole rises to the surface from between the muscle fibers. The capillaries then run longitudinally between the muscle fibers with occasional anastomoses, eventually emptying into venous capillaries and venules, which, except in the region of the tendon, also disappear from view beneath the superficial muscle fibers. Over the surface of the tendon the collecting vessels can often be followed as far as the smaller venules. The capillaries lie loosely in the rather sparse intermuscular connective tissue and tend to slide away as the tip of the pipette is forced against their walls. On this account it was necessary to fix the capillary which was to be pierced by inserting a glass micro-needle into the tissue at the side of the capillary. This needle was pointed in the opposite direction to the pipette and was moved by a Chambers micro-manipulator. After the pipette had pierced the capillary wall the glass needle was removed, or, if necessary, adjusted to prevent blood from flowing through the capillary past the tip of the micro-pipette.

To measure venous capillary pressure the tip of the pipette was inserted into a capillary in the direction of blood flow. The capillary thus pierced acted as a side tube for measuring pressure at the venous ends of the parallel capillaries at the point where they passed into the smallest divisions of the

collecting system. Arterial capillary pressure was determined by directing the pipette toward the division of a terminal arteriole into a group of capillaries. The capillary pressure readings were made as usual by finding the pressure at which fluid flowed neither into nor out of the pipette. This point of equilibrium was reached when the corpuscles in the capillary, though oscillating to and fro with the heartbeat, failed to move consistently toward or away from the tip of the pipette.

In the observations on skin capillaries two series of readings were made. In the first series curare was introduced into the ventral lymph sac, the initial dose of 0.05 cc. of 0.15 per cent solution being reinforced by smaller subsequent doses when necessary. Urethane was used in the second series of experiments in the same dosage as given above, but was introduced into the ventral lymph sac. The web was laid over a triangular glass stage the apex of which fitted between the digits, while the web was spread out loosely upon the surface of the glass. Fine silk threads were tied to the distal ends of the digits in order to keep the web in position on the stage without injuring the tissue itself. The leg was laid out straight to prevent the obstruction of venous flow which is sometimes produced by flexion or by twisting of the leg. The web was irrigated with tap water and illuminated by transmitted light filtered as described above. Since the capillaries of the web are arranged in a freely communicating network the measurement of pressure could be made in much the same way as previously described for the mesenteric capillaries, except for the added difficulty of piercing the skin.

Capillary pressure during hyperemia was studied in muscle after tetanus, which was induced by means of ordinary faradic stimulation through two minute copper electrodes inserted into the lateral border of the longissimus dorsi muscle close to the vertebral column. The electrodes were thus as remote as possible from that portion of tissue which was being observed. The strength of the faradic current was gradually increased by adjusting the secondary coil until the whole muscle was involved in the contraction. The tetanus was continued for $\frac{1}{2}$ to 1 minute and during the resulting hyperemia capillary pressure was determined as described above for the resting muscle.

Hyperemia of the web was produced by stimulation of the axone reflex. The irrigation was stopped and a small crystal of silver nitrate was laid on the moist skin of the web several millimeters from the point at which capillary pressure was being measured. In a few observations the skin was injured instead with a minute cautery.

OBSERVATIONS. *1. Capillary pressure in muscle during rest and after tetanus.* In the surface of carefully exposed resting muscle the rate of blood flow was usually slow and the number of visible capillaries rather small. The blood pressure readings for resting muscle are summarized in table 1

which records in centimeters of water the average pressure in artery, arteriolar capillary, venous capillary, small venule and vein. Arterial pressure was measured in the occipital artery anterior to the cranial attachment of the longissimus dorsi muscle. Average pressure here was approximately the same as that previously observed in the mesenteric artery. Arteriolar pressures are not given since the arterioles could not be reached in this location without grossly disturbing the muscle. The arteriolar capillary pressure readings varied between 11 and 18 cm. water, with an average value of 14.9 cm. and an average deviation of ±1.7 cm. The slight but definite pulsation of corpuscles observed while measuring pressure in this situation indicated that the pulse penetrated to the capillary network. This pulse was so small, however, that it was difficult to measure pulse pressure,

TABLE I

Capillary pressure in frog muscle

		ARTERY (OCCIPITAL)	ARTERIOLAR CAPILLARY	VENOUS CAPILLARY	VENULE	VEIN (CUTANEOUS)
During rest	Number of observations	2	18	39	7	3
	Average pressure, cm. water	35 systolic 22 diastolic	14.9	9.5	8.8	2.2
	Average deviation, cm. water		±1.7	±1.4	±0.7	
	Average diameter, micra		16	24	75	
After tetanus	Number of observations	5	17	12	8	3
	Average pressure, cm. water	37 systolic 22 diastolic	20.1	16.0	9.5	2.5
	Average deviation, cm. water		±2.7	±1.4	±1.9	
	Average diameter, micra		22	26	90	

though by rough estimation it appeared to be about 1 cm. water. The venous capillary pressure readings varied between 7.0 and 12.5 cm. with an average of 9.5 cm. and an average deviation of ±1.4 cm. In the venous capillaries pulsation was extremely slight or entirely absent. Beyond the venous capillary the pressure fell very slowly; in the smaller venules with an average diameter of 75 micra the average pressure was 8.8 cm.

During the period in which the muscle was in tetanus the blood flow through the visible capillaries became slow, irregular and often stopped completely. But immediately after the stimulation ceased and the muscle relaxed, blood flow returned and the tissue became conspicuously hyperemic. The number of visible capillaries was increased, the diameter of individual vessels was obviously greater, and the rate of blood flow was so

rapid that individual corpuscles could no longer be observed as they passed through the capillaries.

It was impossible to measure pressure in the same capillary continuously before, during, and after tetanus since in contraction the movement of the muscle invariably disturbed the relation between the micro-pipette and the capillary. It was necessary therefore to stimulate the muscle and then, after it had relaxed, to introduce the micro-pipette as quickly as possible. The pressure readings in two separate experiments are shown in figure 1, where they have been charted together to save space. The solid line represents pressure in an arteriolar capillary, the dotted line that in a venous

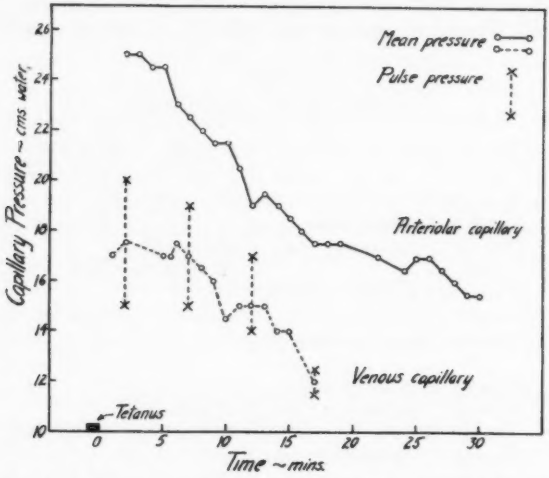

Fig. 1. Chart showing pressure in arteriolar capillary (upper line) and in venous capillary (lower line) of muscle during the hyperemia which followed a one minute period of tetanus.

capillary, during the period of hyperemia. The pressure in both vessels was elevated to a point well outside the range observed in resting muscle. Pulsation was conspicuous in both the arteriolar and venous capillaries, pulse pressure being 4 cm. or more at the time of maximum hyperemia. As shown on the venous capillary pressure line in figure 1, during the period of maximum hyperemia pulse pressure was 5 cm. of water, diminishing with the subsidence of hyperemia to 1 cm. at the end of 17 minutes.

Capillary pressure usually remained at or near the initial high level during the first five minutes of the hyperemia and then rapidly declined as the circulation returned to its usual resting condition. It required from 15 to 20 minutes for capillary pressure, diameter and rate of flow to return

completely to their resting state. After a rest period of 15 to 30 minutes the observation could be repeated with the same result.

In order to express the readings in tabular form the capillary pressures observed during the first five minutes of the hyperemia have been averaged in table 1 to compare with the resting values obtained before the period of tetanus. The arterial pressure showed no significant change. Arteriolar capillary pressure immediately after the period of tetanus averaged 20.1 cm. water with variations from 17 to 26 cm. and an average deviation of ±2.7 cm. The average diameter of the capillaries in hyperemia was 22 micra compared to 15 micra during rest. The rise in pressure penetrated to the venous capillaries where average pressure was 16.0 cm. water, varying between 12.0 and 17.5 cm. with an average deviation of ±1.4 cm. The pressure in the smaller venules was changed very little by the hyperemia.

TABLE 2

Capillary pressure in frog's web

		WITH CURARE		WITH URETHANE	
		Arteriolar capillary	Venous capillary	Arteriolar capillary	Venous capillary
Normal-flow	Number of observations	65	22	71	22
	Average pressure, cm. water	14.5	10.0	13.9	9.6
	Average deviation, cm. water	±1.8	±1.1	±1.7	±0.6
	Average diameter, micra	17	24	18	25
Hyperemia of axone reflex	Number of observations			27	12
	Average pressure, cm. water			19.5	16.5
	Average deviation, cm. water			±2.9	±0.5

The rather remote possibility that the rise in capillary pressure might be secondary to a generalized change in venous pressure has been ruled out by measuring the pressure in the vena cutanea magna during rest and after stimulation of the longissimus dorsi muscle. There is no significant change in general venous pressure as indicated in the last column of table 1. This indicates that the rise in capillary pressure must be referred to arteriolar dilatation.

2. Capillary pressure in the web. Cutaneous capillary pressure was determined first in the webs of ten curarized frogs. The web vessels in two of these animals showed without obvious cause a conspicuous hyperemia: the skin of the legs was pinker than normal and flow through the web vessels was as rapid as that seen during stimulation of the axone reflex. The readings obtained in these two animals have been excluded from the normal series which is summarized in table 2. With normal circulation arteriolar capillary pressure varied between 10.0 and 20.5 cm. water with an average

of 14.5 cm. and an average deviation of ±1.8 cm. Venous capillary pressure varied between 8.5 and 15.5 cm. water, with an average of 10.0 cm. and an average deviation of ±1.1 cm.

A second series of readings was made in frogs under urethane anesthesia to determined whether the stoppage of the lymph hearts by curare had any conspicuous influence on capillary pressure. Arteriolar capillary pressure in these animals varied from 10.0 to 19.0 cm. water with an average of 13.9 cm. and an average deviation of ±1.7 cm. Venous capillary pressure varied between 8.5 and 13.0 cm. water, with an average of 9.6 cm. and an average deviation of ±0.6 cm. When the readings in these two series are

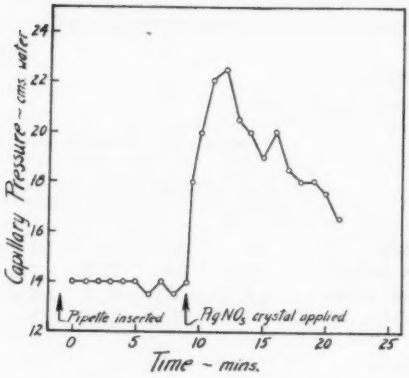

Fig. 2

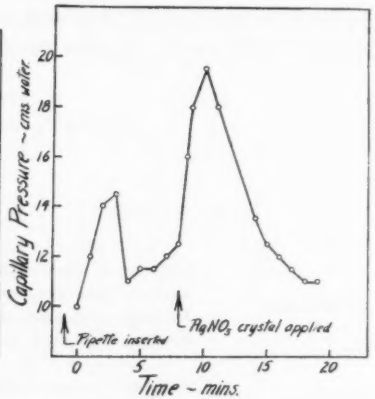

Fig. 3

Fig. 2. Chart showing pressure in arteriolar capillary of web during stimulation of the axone reflex. The rise in pressure followed injury of the skin by application of a crystal of silver nitrate.

Fig. 3. Chart showing pressure in venous capillary of the web during stimulation of the axone reflex. The initial, smaller rise in pressure followed the insertion of the pipette; the second larger rise in pressure followed injury of the skin by the application of a crystal of silver nitrate.

examined statistically it becomes evident that no significance can be attached to the difference in the readings obtained with curare and with urethane.

When capillary pressure was measured repeatedly over a period of 5 to 10 minutes from the piercing of the skin and capillary, the level of pressure in many instances remained unaltered as shown in the first portion of figure 2. In about half the determinations, however, the pressure rose quickly during the first 1 to 3 minutes, to a point from 0.5 to 4.5 cm. above the original reading, then fell again approaching the original level by the third to the fourth minute, as shown in figure 3. This resembles in its occurrence and

time relations the temporary rise of pressure observed in human cutaneous capillaries under similar conditions (Landis, 1930). It would appear from this that in the frog also the local injury produced by piercing the skin in some instances was not severe enough to stimulate the axone reflex, whereas in other instances the sensory nerve endings were stimulated and there resulted a transient rise of pressure. In order to eliminate as far as possible any residual effects of the initial trauma, pressure determinations were repeated until a relatively constant value was reached.

During the hyperemia produced by placing a crystal of silver nitrate on the skin of the web pressure rose in both the arteriolar and the venous capillaries. Figure 2 indicates the change in an arteriolar capillary and figure 3 in a venous capillary. The rise in pressure was rapid, reaching a maximum within 1 to 3 minutes, and returning in most instances to the resting level within 7 to 20 minutes. Occasionally the hyperemia continued and capillary pressure failed to fall, so that at the end of 20 minutes or more the pressure was still above the previous resting level. This was due probably to the continued spread of the silver nitrate to new and previously unaffected areas of the skin. When the axone reflex was stimulated by a cautery this long sustained elevation of pressure was observed less frequently.

Pulse pressure was clearly increased throughout the capillary network, and amounted usually to about 2 cm. water. The changes in pressure were accompanied with great faithfulness by corresponding changes in the rate of blood flow. In a few instances the crystal of silver nitrate failed to produce hyperemia in the field under observation and in these instances capillary pressure failed to rise.

The maximum pressures observed during the hyperemia have been averaged for comparison with the resting figures. Thus during the stimulation of the axone reflex arteriolar capillary pressure varied from 14.0 to 26.5 cm. water with an average of 19.5 cm. and an average deviation of ± 2.9 cm. Venous capillary pressure varied from 15.0 to 17.5 cm. with an average of 16.5 cm. and an average deviation of ± 0.5 cm. Hyperemia therefore produces a significant rise of pressure throughout the capillary network of the frog's web, but this rise is not quite so great as that observed in the hyperemia of muscle.

3. Capillary pressure, venous pressure, and the renal portal system. The capillary pressure readings made in mesentery, web, and muscle are compared in table 3. There is a striking similarity in the level of average capillary pressure in the three situations during rest and to a certain extent also during hyperemia. The capillaries of the mesentery and web, however, are separated from the heart by portal systems, whereas the muscle capillaries have no such secondary resistance between them and the heart. It seemed strange that with this difference capillary pressure in the three situations should be so similar.

To estimate the magnitude of the secondary resistance offered to the blood returning from the web capillaries, venous pressure was measured simultaneously in the external iliac vein and in the vena cutanea magna. One cannula was introduced into the proximal portion of the femoral vein, the other into a large terminal ramification of the vena cutanea magna. The two cannulae were connected through a two-way stopcock to the manometer previously used for determining capillary pressure. The whole system was filled with salt solution to which had been added a small amount of heparin. The manometer was calibrated in order to verify the zero read-

TABLE 3

Summary of capillary pressure readings in the frog

TISSUE		AVERAGE BLOOD PRESSURE		AVERAGE LENGTH OF CAPILLARY NET WORK	TREATMENT OF ANIMAL
		Arteriolar capillary	Venous capillary		
		cm. water	*cm. water*	*mm.*	
Normal circulation	Mesentery	14.4	10.1	1.50	Pithed
	Muscle	15.2	9.5	0.76	Urethane
	Skin (web)	14.5	10.0	0.85	Curare
		13.9	9.6		Urethane
Hyperemia	Muscle	20.1	16.0		Urethane
	Skin (web)	19.5	16.5		Urethane

TABLE 4

Pressures in the vena iliaca externa and in the vena cutanea magna

OBSERVATION NUMBER	VENOUS PRESSURE		DURATION OF EXPERIMENT
	Vena iliaca externa	Vena cutanea magna	
	cm. water	*cm. water*	*minutes*
1	2.5–5.5	1.0–4.0	50
2	2.3–5.0	1.7–2.5	37
3	3.2–3.5	1.4–1.5	10

ing and to correct for any difference between the level of the animal and that of the manometer. The pressures were measured alternately in the two veins by lowering the column of water in the manometer until blood entered the cannula. The pressure in the system was then gradually raised until equilibrium was reached. By observing the movement of the blood in the cannula through a binocular microscope the amount of fluid injected into the animal was reduced to a minimum. The pressures observed in 3 experiments are shown in table 4.

Pressure in the iliac vein was always higher than that in the vena cutanea

magna, the greatest difference observed being 3.5 cm. water. The majority of pressure readings differed only by 1.0 to 2.0 cm. water. This is in agreement with the more extensive series of observations by Hayman (1928), who found that the average pressure in the renal portal vein was 5.0 cm. water, while that in the renal vein was 2.6 cm.

Discussion. The primary purpose of these studies was to ascertain whether hyperemia was accompanied by a significant rise in capillary pressure. The normal pressure relations in the frog are probably expressed most accurately in the figures obtained from the web since in this location an abnormal exposure of the capillaries was not required. In the muscle the amount of disturbance was reduced as much as possible, but even so the circulation probably cannot be regarded as completely normal. Nevertheless the rate of blood flow in the capillaries of the exposed and resting longissimus dorsi muscle was not outside the range usually described as normal. In both tissues capillary pressure was significantly higher during hyperemia.

The results are in general agreement with the previous studies of capillary pressure in the mesentery, in that the fall in pressure does not cease at the junction of the capillaries and arterioles but continues into the capillary network. This fall in pressure, amounting to about 4 cm. water, indicates that the peripheral resistance to blood flow in the general body tissues, as well as in the mesentery, resides partly in the arterioles and partly in the capillaries. The share contributed by the capillaries under usual resting conditions is rather small, but in hyperemia arteriolar relaxation diminishes the resistance proximal to the capillaries; capillary pressure is elevated and the proportion of peripheral resistance which is situated in the capillary network is increased.

The fall in pressure is approximately the same in the three tissues though, as shown in table 3, the average distance between the points at which the arteriolar and venous capillary pressures were determined is slightly less in muscle and web than in the mesentery, where not infrequently single capillaries are over two millimeters long. Even in muscle and skin, however, the distance is greater than the usually quoted figure of 0.5 mm., because the capillary network as a whole consists of two to many series of single capillaries arranged end to end. In general the muscle capillaries, though shorter, have a smaller diameter than those of the mesentery, which would tend to equalize the pressure fall in the two regions. In the web the capillary network is composed of a very close meshwork of short capillaries, so that comparison with the other two systems is difficult, but here also the pressure difference between the two ends of the complete network was approximately the same as in the other two regions studied.

The influence which the portal systems play in determining the level of systemic capillary pressures is practically imperceptible in the capillary

pressure measurements here reported. According to Churchill, Nakazawa and Drinker (1927), "It is almost certain that the existence of a secondary capillary bed must act as an important factor in the determination of the pressure gradient in the vessels of the primary circuit." This would be true if the secondary resistance were structurally a capillary bed. It appears, however, that the renal portal system is made up of a series of short capillary sinusoids of rather large diameter. Hayman (1928) mentions their shortness in describing the changes in form exhibited by small globules of mercury injected into the renal portal vein. As the mercury passed from the renal portal vein into the capillary sinusoids the globules first elongated and then divided into smaller globules about 25 to 50 micra in diameter. These could be followed into the renal vein where they formed large globules again. Shore (1901) from his embryological and histological studies regarded the intertubular blood vessels as venous sinuses rather than true capillaries, and this view is shared by more recent investigators. The large diameter and the reduced length of these vessels would tend to lessen the resistance to flow and would explain the relatively slight fall in pressure in the renal portal system. The capillary system of the liver is also referred to as "sinus-like" by Shore (1901) and the connections of the hepatic portal and the renal portal circulations are such that the pressures in these two regions must be very similar.

The pressure fall in the renal portal system is small, usually about 2.0 cm. water. Such a difference in venous pressure could hardly be expected to cause a significant difference in venous capillary pressure, particularly since the latter is maintained at an absolute level at least twice as high as that in the iliac and cutaneous veins. The slight pressure difference is apparently equalized in the larger veins since the pressures in the venules of the mesentery and of the muscle are 7.5 and 8.8 cm. respectively.

The information concerning capillary pressure in muscle and skin is of interest in view of the abundant production of lymph which has been described as the usual condition in the toad by Isayama (1924) and in the frog by Conklin (1930a). According to the former in 24 hours there is filtered through the capillary walls an amount of fluid equal to fifty times the plasma volume. Conklin (1930a) found the amount exceedingly variable. Cauterization of the two posterior lymph hearts was followed in one instance by the loss of fluid from the blood at a calculated rate of 62 cc. in 24 hours, while in another instance there was no loss whatever. Curarization, which stopped all four lymph hearts, caused body weight of frogs immersed in water to increase by 16 per cent to 28 per cent in the course of 6 hours.

Equally conspicuous is the variability in the reported readings of the osmotic pressure of the plasma proteins in the frog. The four available average figures are 5.7 cm. water (Krogh, 1922), 10.5 cm. (White, 1924),

11.5 cm. (Landis, 1927), and 7.1 cm. (Churchill, Nakazawa and Drinker, 1927). The colloid osmotic pressure readings for single normal animals range from 5.5 cm. water (Krogh, 1922) to 13.4 cm. (Churchill, Nakazawa and Drinker, 1927).

It has been shown (Landis, 1927) that in the frog mesentery the rate at which fluid moves through the capillary wall depends on the balance between capillary pressure and the effective osmotic pressure of the plasma proteins. Thus with a colloid osmotic pressure in the vicinity of 13 cm. water, and an average capillary pressure of 12 cm., the balance will favor absorption except in situations, such as the skin (Churchill, Nakazawa and Drinker, 1927; Conklin, 1930b), where leakage of protein lowers the effective colloid osmotic pressure of the plasma proteins. With a colloid osmotic pressure of 5.5 cm., however, the balance will overwhelmingly favor filtration everwhere, and the amount of lymph produced under these circumstances would be very considerable. In hyperemia the rise of capillary pressure would accelerate the rate still further, or cause filtration where the balance under usual conditions favored absorption. This highly variable relation between capillary pressure and the osmotic pressure of the plasma proteins aids in explaining not only the magnitude, but also the variability, of lymph production in the frog.

SUMMARY

The capillary blood pressure in the muscles and skin of the frog was studied by means of the micro-injection method, during average circulation and during hyperemia.

In resting muscle average arteriolar capillary pressure was 14.9 cm. water, average venous capillary pressure 9.5 cm. During the hyperemia which followed tetanus average arteriolar capillary pressure was 20.1 cm. and average venous capillary pressure 16.0 cm., without significant pressure change in arteries, venules and the vena cutanea magna. Pulse pressure in the capillary network was increased during the period of hyperemia.

In the webs of frogs under urethane average arteriolar capillary pressure was 13.9 cm., and average venous capillary pressure 9.6 cm. The hyperemia produced by stimulating the axone reflex was accompanied by a rise in arteriolar capillary pressure to an average of 19.7 cm., and in venous capillary pressure to an average of 16.5 cm.

As in the mesentery the fall of pressure in the peripheral vessels of muscle and skin does not cease abruptly at the junction of the arterioles and capillaries, but continues to the venous capillary before flattening. This indicates that the peripheral resistance to blood flow is situated partly in the capillary network.

The fall of pressure in the renal portal circulation varied between 1.0 and 3.5 cm. water. There was no evidence that this small secondary resistance

produced any significant difference between the pressure gradients observed in the capillaries of the web and the longissimus dorsi muscle.

The capillary pressure readings in muscle and skin are discussed with reference to lymph production in the frog.

It is a pleasure to express my gratitude to Professor Krogh for suggesting this subject, and for much helpful advice during its investigation.

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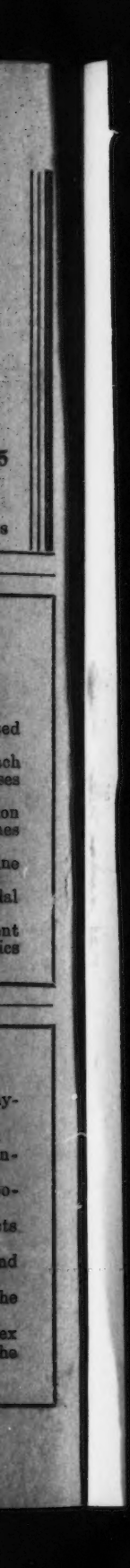
Tentative Contents of Volume XII, 1932

F. C. Koch: The Male Sex Hormone
A. D. Hirschfelder and R. N. Bieter: Local Anesthetics: Relation of Constitution to Action
H. E. Roaf: Color Vision and Its Defects
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K. S. Lashley: On Cerebrum Localization
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